

# Four new species of troglomorphic *Coecobrya* Yosii, 1956 (Collembola, Entomobryidae) from Thailand based on morphological and molecular evidence, with an updated key of Thai troglomorphic species

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## Abstract

Four new species of troglomorphic *Coecobrya* Yosii, 1956 are described from caves located in the central and northeastern regions of Thailand. *Coecobrya whitteni* **sp. nov.** and *C. troglobia* **sp. nov.** are from Khon Kaen province, *C. ellisi* **sp. nov.** is from Phetchabun province and *C. phitsanulokensis* **sp. nov.** is from Phitsanulok province. They all exhibit remarkable troglobitic characters i.e. elongated antennae, legs and furca, slender claw complex and large body size. *Coecobrya whitteni* **sp. nov.** is similar to *C. troglobia* **sp. nov.** and they were found a distance of only 3.4 km from each other. However, they are mainly different in the number of An mac on dorsal head and number of chaetae of Th. II. Likewise, *C. ellisi* **sp. nov.** is similar to *C. phitsanulokensis* **sp. nov.**. However, they differ in the number of An mac on the dorsal head, the number of central mac on Abd. II, central mac on Abd. IV, lateral mac on Abd. IV and the number of inner teeth of the claw. Moreover, *C. ellisi* **sp. nov.** has orange pigment dots on the body, a unique character, considering that all other troglomorphic *Coecobrya* species in Thailand are devoid of pigmentation. The results of the molecular approach based on two partial mitochondrial markers (COI and 16S rDNA) and a nuclear gene fragment (28S rDNA) supported the results of morphological species discrimination in separating the four nominal populations as valid species. An updated dichotomous key of Thai troglomorphic *Coecobrya* species is also given.



**Keywords**

DNA barcoding, Entomobryinae, molecular phylogeny, subterranean habitat, troglomorphy

**Introduction**

The subterranean genus *Coecobrya* Yosii, 1956 is globally distributed, especially in Japan, southern China and Thailand (Lukić 2019). They are characterized by the absence of body scales, labral papillae and dental spines, medium size (0.9–2.5 mm), polymacrochaetotic chaetotaxy, four antennal segments without apical bulb, body pigmentation reduced or absent, reduction of eyes (with 0+0 to 3+3 eyes), furca with falcate mucro and a basal spine (Deharveng 1990; Zhang et al. 2011a; Jantarit et al. 2019). To date, 70 species have been described worldwide (Bellinger et al. 1996–2021).

In Thailand, the genus *Coecobrya* is common and widespread throughout the country, especially in cave environments. So far 15 species (21 % of the worldwide total) are reported from the country, of which 13 species are found exclusively in cave habitats. The majority of them (11 species) are described from caves in the southern region and have a narrow range of dispersion. They are: *C. cavicta* Nilsai & Zhang, 2017; *C. chumphonensis* Zhang & Nilsai, 2017; *C. donyoo* Zhang & Jantarit, 2018; *C. khaopacla* Zhang & Jantarit, 2018; *C. khromwanaramica* Zhang, 2018; *C. polychaeta* Zhang & Nilsai, 2017; *C. phanthuratensis* Zhang & Jantarit, 2018; *C. promdami* Zhang & Jantarit, 2018; *C. ranongica* Nilsai & Zhang, 2018; *C. sirindhornae* Jantarit, Satasook & Deharveng, 2019; *C. specusicola* Zhang & Nilsai, 2018 (Nilsai et al. 2017; Jantarit et al. 2019; Zhang et al. 2018a). Three other species are described from Chiang Mai, northern Thailand: *C. guanophila* Deharveng, 1990 from a cave; *C. similis* Deharveng, 1990 from a cave and epigeal habitats; *C. lanna* Zhang, Deharveng & Chen, 2009 from forest litter. Another species, *C. cf. hoefti* (Schäffer, 1896), was recorded from Chiang Mai (Northern Thailand) and Khon Kaen (northeastern Thailand) in the soil of mixed dry deciduous forests (Deharveng 1990; Zhang et al. 2009; Jantarit et al. 2016). Many species of the genus have been collected and remained undescribed (Jantarit et al. 2016).

Troglomorphic characters in the Collembola are elongated appendages (antennae, legs and furca), elongated and slender claw complex, pointed tenent hair, large body size, multiplication of antennal chaetae, blindness and depigmentation (Christiansen 2012; Deharveng et al. 2018; Lukić et al. 2018; Lukić 2019; Jantarit et al. 2019). In Thai caves the genus *Coecobrya* displays various degrees of troglomorphy, from a small set of morphological modifications to a large degree of darkness adaptation (Deharveng 1990; Nilsai et al. 2017; Zhang et al. 2009, 2018a; Jantarit et al. 2019). Two morphological forms are currently recognized in Thai caves and both forms have narrow ranges. The first form resembles that of the epigeal species with short antennae and appendages, short and rather swollen claw morphology and small size. It is always associated with eutrophic habitats in large populations, especially on bat guano, and it is never troglomorphic (Deharveng 1990; Zhang et al. 2018a). The second form possesses long to extremely elongated appendages (antennae, legs and furca), slender



claw morphology and larger body size, which are considered as troglomorphic to highly troglomorphic adaptations (Deharveng and Bedos 2000; Nilsai et al. 2017; Jantarit et al. 2019). *Coecobrya sirindhornae*, for example, belongs to the second form and is the most highly troglomorphic Collembola in Southeast Asia and even exhibits stronger troglomorphic characters than the cave-adapted species from the temperate region, especially the elongation of antennae (Jantarit et al. 2019). All the troglomorphic species are rare with rather small populations and are generally linked to oligotrophic habitats in the dark zone of caves with a typically wet and moist environment. However, the troglomorphic species are reported only from southern Thailand, with four described species so far. These are *C. cavicta*, *C. polychaeta* and *C. sirindhornae* from Satun province and *C. chumphonensis* from Chumphon province.

During our sampling surveys in the limestone caves of Thailand several troglomorphic, occasionally highly troglomorphic, *Coecobrya* species were seen and collected in several areas of Thailand. Four of them are described in this work and they were found exclusively in caves of the central region (Phetchabun and Phitsanulok provinces) and in the western part of the northeastern region in Khon Kaen province. They are therefore the first described cave *Coecobrya* species from the central and northeastern regions of Thailand. In the last part of this work an updated key of the Thai troglomorphic *Coecobrya* species is also given.

## Methods

### Taxa sampling and morphological identification

Specimens were collected with entomological aspirators and stored in 95% ethanol. They were later kept in a freezer at  $-20^{\circ}\text{C}$ . Specimens were cleared in Nesbitt solution, heated at  $60^{\circ}\text{C}$  for 1–2 minutes, before mounting on glass slides using Marc André II solution. Morphological characters were examined using an Olympus BX 51 microscope with phase-contrast and a drawing tube. Illustrations were enhanced with Adobe Illustrator CC/PC for Windows (Adobe Inc.). Photographs were taken with a Canon 5D digital camera using a Canon MP-E 65 mm Macro Photo Lens and Canon Extender EF 2.0 $\times$  III (Canon, Tokyo, Japan) and a Stack-Shot Macrorail (Cognisys Inc, MI, USA). Photos were then combined in Helicon Focus 6.8.0 (Helicon Soft, Ukraine). The map was taken and modified from Mitrearth (<http://www.mitrearth.org/>). Photographs were improved later using Adobe Photoshop CC/PC for Windows (Adobe Inc.).

### DNA extraction, amplification and sequencing

The DNA of each individual was isolated from the whole body using a DNeasy Tissue Extraction Kit (Qiagen, Hilden, Germany), following the manufacturer's protocols. Fragments of the mitochondrial genes Cytochrome Oxidase subunit I (COI), 16S rRNA (16S) and nuclear gene 28S rRNA were amplified using the primers listed in Suppl. material 2, Table S1. Thermocycler settings for each marker follow the refer-



ences provided in Suppl. material 2, Table S1. The PCR mixture (50 µL) contained 1× PCR Buffer, 0.2 mM dNTP mixture, 1.5 mM MgCl<sub>2</sub>, 0.25 µM of each forward and reverse primer, 1× CoralLoad Concentrate, 1.25 units TopTaq DNA polymerase and ≈15 ng DNA template. All amplifications were verified via gel electrophoresis using a 1% agarose gel stained with SYBR Safe DNA Gel Stain (Thermo Fisher Scientific, CA, USA) before sending to the First Base Laboratories Sdn. Bhd. (Selangor, Malaysia) and Macrogen, Inc. (Seoul, South Korea) for sequencing. All new sequences are deposited in GenBank (Suppl. material 2, Table S2).

## Data analysis

The data set includes 102 sequences generated from the 57 specimens and 55 published sequences from GenBank. Overall the data set consists of 46 sequences of COI from 14 species, 28 sequences of 16S rDNA from 12 species, and 28 sequences of 28S rDNA from 12 species (Suppl. material 2, Table S3). All sequences of each gene were aligned using Clustal Omega version 1.1.0 (Sievers and Higgins 2014) implemented in SeaView version 5.0.4 (Gouy et al. 2021). Pairwise genetic distance of each sample for each gene was calculated using p-method in Mega version 10.2.5 (Kumar et al. 2018). A concatenated supermatrix (1,478 bp) consisting of the three genes (COI, 16S and 28S) was created manually. The substitution models under the corrected Akaike information criterion of 16S and 28S genes and the three codon positions of COI gene were accessed using ModelFinder (Kalyaanamoorthy et al. 2017). *Willowsia nigromaculata* (Lubbock, 1873) was used as an outgroup. Bayesian phylogenetic inference of the concatenated supermatrix was generated using BEAST version 2.6.6 (Bouckaert et al. 2019). All *Cocobrya* sequences were grouped as a monophylum to create *W. nigromaculata* as an outgroup. With the same partitioning scheme as the maximum likelihood method, the optimal substitution model was chosen using bModelTest version 1.2.1 (Bouckaert et al. 2017). Trees and clock models were linked across all partitions. Strict molecular clock and Yule tree prior were used. The analysis was performed for 10 million Markov chain Monte Carlo generations and sampled every 1,000 generations. Tracer version 1.7.2 (Rambaut et al. 2018) was used to analyze the posterior estimate quality by removing 10% burn-in, and the maximum clade credibility tree was obtained from TreeAnnotator version 2.6.6 (Bouckaert et al. 2019). Also, phylogenetic trees of each gene inferred by Maximum Likelihood and for the concatenated supermatrix were created using IQ-Tree version 2.1.4-beta (Minh et al. 2020) with 1,000 ultrafast bootstraps.

## Terminology

The pattern of labial chaetotaxy follows Gisin's system (1967) and Zhang and Pan (2020), with upper-case letters for ciliated and lower-case letters for smooth chaetae. We follow Zhang et al. (2016) for the clypeal chaetotaxy and Fjellberg (1999) for labial palp. Postlabial chaetotaxy and Antennae III organ were described following Chen and Christiansen (1993) and Jantarit et al. (2019). The number of dorsal macrochaetae



from Th. II–Abd. IV is given by half-tergite and the description follows Szeptycki (1979), Zhang et al. (2011b) and Jantarit et al. (2019). The S-chaetae system was modified from Zhang and Deharveng (2015). All type material is deposited in the collections of the Princess Maha Chakri Sirindhorn Natural History Museum (NHM-PSU), Prince of Songkla University, Thailand.

### Abbreviations used in the description

<b>Ant. I–IV</b>	antennal segment I–IV;
<b>Abd. I–VI</b>	abdominal segment I–VI;
<b>Gr.</b>	cephalic group;
<b>mac</b>	macrochaeta(e);
<b>mes</b>	mesochaeta(e);
<b>mic</b>	microchaeta(e);
<b>ms</b>	S-microchaeta(e)/microsensillum(a);
<b>psp</b>	pseudopore(s)
<b>s</b>	ordinary S-chaeta(e)/sens;
<b>tita</b>	tibiotarsus.
<b>tric</b>	trichobothrium(ia);
<b>Th. I–III</b>	thoracic segment I–III.

## Results

### Taxonomic part

**Class Collembola** Lubbock, 1873

**Order Entomobryomorpha** Börner, 1913

**Family Entomobryidae** Schäffer, 1896 *sensu* Zhang et al, 2019

**Subfamily Entomobryinae** Schäffer, 1896 *sensu* Zhang and Deharveng 2015

**Genus *Coecobrya*** Yosii, 1956

***Coecobrya whitteni* Nilsai & Jantarit, sp. nov.**

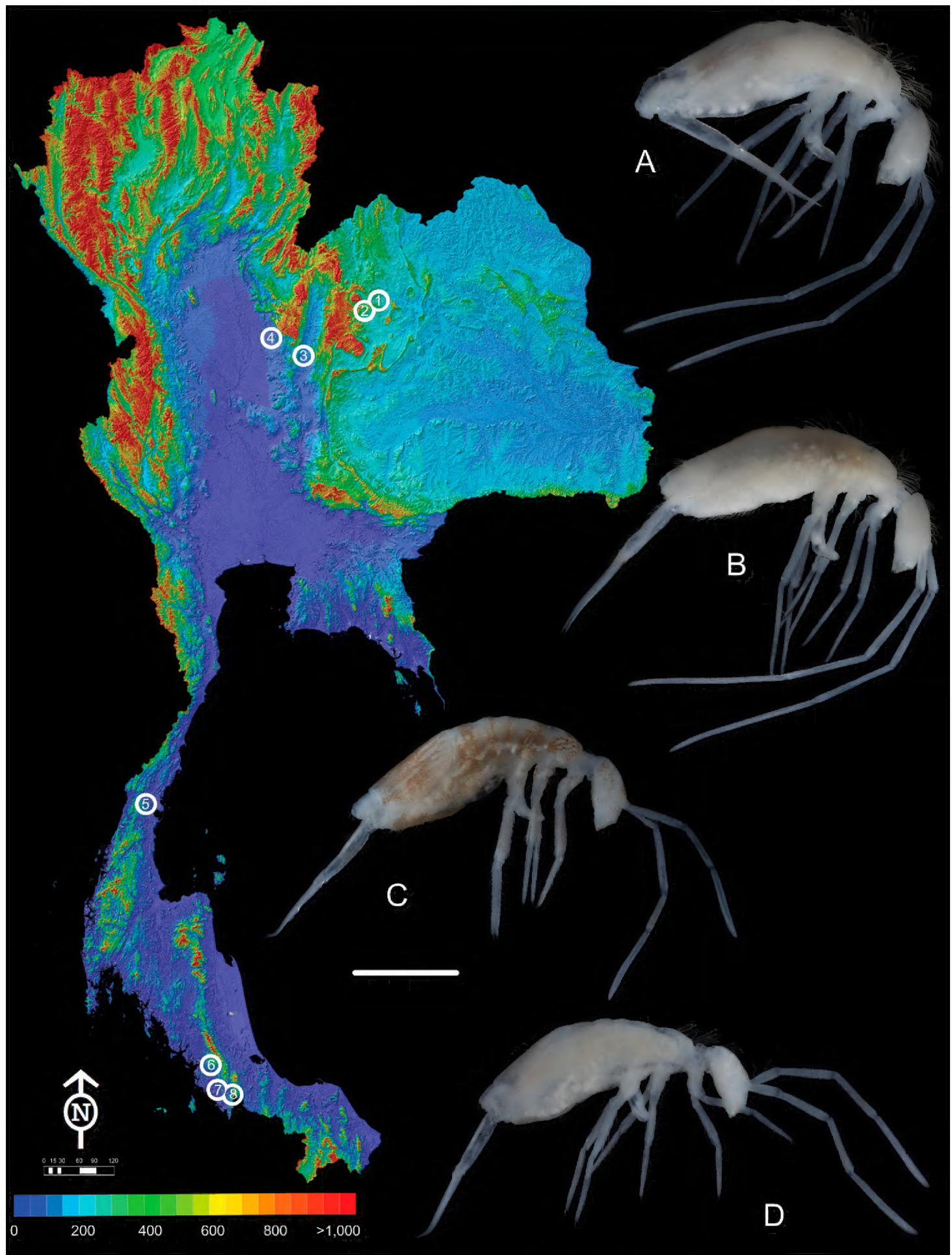
<http://zoobank.org/84FBEE6D-88C3-459F-B8D7-1B8A741D7393>

Figures 1A, 2–4, Table 1

**Type material. *Holotype*:** female on slide. Thailand, Khon Kaen province, Chum Phae district, Tham Nayn Noi (note: “tham” = “cave” in Thai), altitude 359 m a.m.s.l., 16.8292°N, 101.9848°E. 3.XII.2020; S. Jantarit, A. Nilsai, K. Sarakhamhaeng and K. Jantapaso leg. (sample # THA\_SJ\_KKN04), dark zone of a cave, by entomological aspirator. ***Paratypes*:** same data as holotype, 11 specimens (three female and eight subadults on slides). ***Additional material*:** same data as holotype, 3 specimens (in ethanol)

Holotype and 11 paratypes on slides deposited in NHM-PSU.





**Figure 1.** The distribution map of the troglomorphic *Coecobrya* in Thailand and the habitus of the four new species. (1) **A** *Coecobrya whitteni* sp. nov. (2) **B** *C. troglobia* sp. nov. (3) **C** *C. ellisi* sp. nov. (4) **D** *C. phitsanulokensis* sp. nov. (5) *C. chumphonensis* Zhang & Nilsai, 2017 (6) *C. sirindhornae* Jantarit, Satasook & Deharveng, 2019 (7) *C. polychaeta* Zhang & Nilsai, 2017 and (8) *C. cavicta* Nilsai & Zhang, 2017. Scale bar: 1 mm.



**Table 1.** Comparison of troglomorphic *Coecobrya* in Thailand: *C. chumphonensis* Zhang & Nilsai, 2017, *C. cavicta* Nilsai & Zhang, 2017, *C. polychaeta* Zhang & Nilsai, 2017, *C. sirindhornae* Jantarit, Satsook & Deharveng, 2019, *C. nupa* Christiansen & Bellinger, 1992. *C. whitteni* Nilsai and Jantarit sp. nov., *C. troglobia* Jantarit and Nilsai sp. nov., *C. ellisi* Jantarit and Nilsai sp. nov., *C. phitsanulokensis* Jantarit and Nilsai sp. nov. Key: c = ciliated chaetae, s = smooth chaetae, ? = not given in literature description. Characters indicated in parentheses are rarely observed.

Characters/species		<i>C. chumphonensis</i>		<i>C. cavicta</i>	<i>C. polychaeta</i>		<i>C. sirindhornae</i>		<i>C. nupa</i>	<i>C. ubitteni</i> sp. nov.	<i>C. troglobia</i> sp. nov.		<i>C. ellisi</i> sp. nov.	<i>C. phitsanulokensis</i> sp. nov.
Body length		up to 2.82 mm		1.72 mm	up to 2.58 mm	up to 2.6 mm	2.0 mm	1.8–2.3 mm	2.0–2.4 mm	1.6–2.1 mm	1.8–2.3 mm			
Ant./head ratio		3.70–4.48		2.67	5.91–7.12	8.0–12.3	6.5	3.78–5.14	4.0–6.8	3.5–6.4	3.1–4.2			
Long smooth straight chaetae on Ant. I		absent		present	absent	present	?	present	present	present	present			
No. of paddle-like chaetae on Ant. II		2–4		?	1	10–12	?	not seen	not seen	not seen	not seen			
Number of chaetae on clypeus														
Prefrontal area		3s		?	3s	3s	?	3s	3s	3s	3s			
Facial area		8s		?	2s; 7–10c	2s; 7–10c	?	2s; 6c	2s; 4c	2s; 5c	2s; 4–7c			
Number of chaetae on dorsal head														
An area		2		4	4	1	?	4	5	3	5			
M series		4		3	3	0	?	3	3	3	3			
Group II (Gr. II)		6(7)		4	3	1	?	4	4	4(5)	4(5)			
A0		mac		mac	mic	mes	?	mac	mac	mac	mac			
Number of sublobal hairs on maxillary outer lobe		3		3	4	4	3	3	3	4	4			
Lateral process of labial palp		short		short	long	long	short	long	long	long	long			
Labial chaetae shape		mRelI <sub>2</sub>		mrelI <sub>2</sub>	M <sub>1</sub> m <sub>2</sub> relI <sub>2</sub>	m <sub>1</sub> m <sub>2</sub> relI <sub>2</sub>	M <sub>1</sub> m <sub>2</sub> relI <sub>2</sub>	mrelI <sub>2</sub>	mrelI <sub>2</sub>	m(M) <sub>1</sub> m <sub>2</sub> relI <sub>2</sub>	m <sub>1</sub> m <sub>2</sub> relI <sub>2</sub>			
Postlabial chaetae X		minute		minute	normal	normal	?	minute	minute	normal	minute			
Number of chaetae along cephalic groove		4s; 5–7c		3–4s; 7–8c	6–7s; 5–10c	7–9s; 3–7c	?	5–6s; 1–2c1–2mics	6s; 1c; 2mics	2–5s; 1–5c; 2mics	4–7s; 1–2c; 1–2(3–4)mics			
Chaetotaxy of Th. II														
Number of medio-medial mac		4–6		3	7(6)	3	2	2	3	3	3			
Number of medio-sublateral mac		3		3	4	4	3	1	2	3	3			
Number of posterior mac		25–32		30–31	29–40	15–18	15?	18–20	15–18	28–31	31–33			
Number of mac on Th. III		32–35		35	35–43	32–35	15?	23–26	24–27	29–36	32–33			
Number of mac on Abd. I		6–7		6–7	8–9	6	4	6	6	7	7			
Number of central mac on Abd. II		3		3	4(3)	2	2	2	2	2	3(4)			
Chaetotaxy of Abd. III														
Number of central mac		2		1	1	1	1	1	1	2	2			
Number of lateral mac		3		3	3	3	2?	3	3	3	3			

Characters/species	<i>C. chumphonensis</i>		<i>C. cavicta</i>		<i>C. polychaeta</i>		<i>C. sirindhornae</i>		<i>C. nupa</i>		<i>C. whitteni</i> sp. nov.		<i>C. troglobia</i> sp. nov.		<i>C. ellisi</i> sp. nov.		<i>C. phitsanulokensis</i> sp. nov.	
	ms	not seen	not seen	present	present	not seen	not seen	?	not seen	not seen	not seen	not seen	not seen	present	present	present	present	present
Chaetotaxy of Abd. IV																		
Number of central mac		7	7–9	6	6	6	4	5	5	5	8	9						
Number of lateral mac		10–12	11	9	8	?	6	6	6	6	9	8						
Ventral tube chaetae																		
anterior face		9–12c	?	12c	10c	?	6–8c	7–8c	6–7c	6–7c								
posterior face		13	?	20–31	20–30	7	8–13	14–18	12–13	12–13								
lateral flap		7(10)	?	9–12	7–8s (5c)	6	6–7s (1c)	7–9s (1–2c)	6–7s; 1–3c	6–7s; 1–3c								
Tenent hair shape		usually pointed	pointed	pointed	pointed	pointed	clavate/pointed	clavate/pointed	pointed	pointed								
Number of ungual inner teeth		3	2	3	3	3	3	3	3	3	3	2						
Unguiculus outer edge shape		serrate	serrate	serrate	2–3 teeth	?	serrate	serrate	?	serrate								
Smooth chaetae on trochanteral organ		12–22	15–16	15–25	12–18	16	18–21	19–23	17–23	17–23								
Chaetae on manubrial plaque		4–7	4	4–10	3	5	3–6c	5c	5–6c	5–6c								
Chaetae on ventrodiscal part of manubrium		11–15c	13c	15–25c	8–10c	?	13c	?	10–12c	13+13c								
Mucronal spine		nearly reaching mucronal apex	nearly reaching mucronal apex	nearly reaching mucronal apex	nearly reaching mucronal apex	beyond mucronal apex	nearly reaching mucronal apex	nearly reaching mucronal apex	nearly reaching mucronal apex	nearly reaching mucronal apex								
			reaching mucronal apex															
Locality																		
		Chumphon, Thailand	Satun, Thailand	Satun, Thailand	Satun, Thailand	Maui, Hawaii	Khon Kaen, Thailand	Khon Kaen, Thailand	Phetchabun, Thailand	Phitsanulok, Thailand								



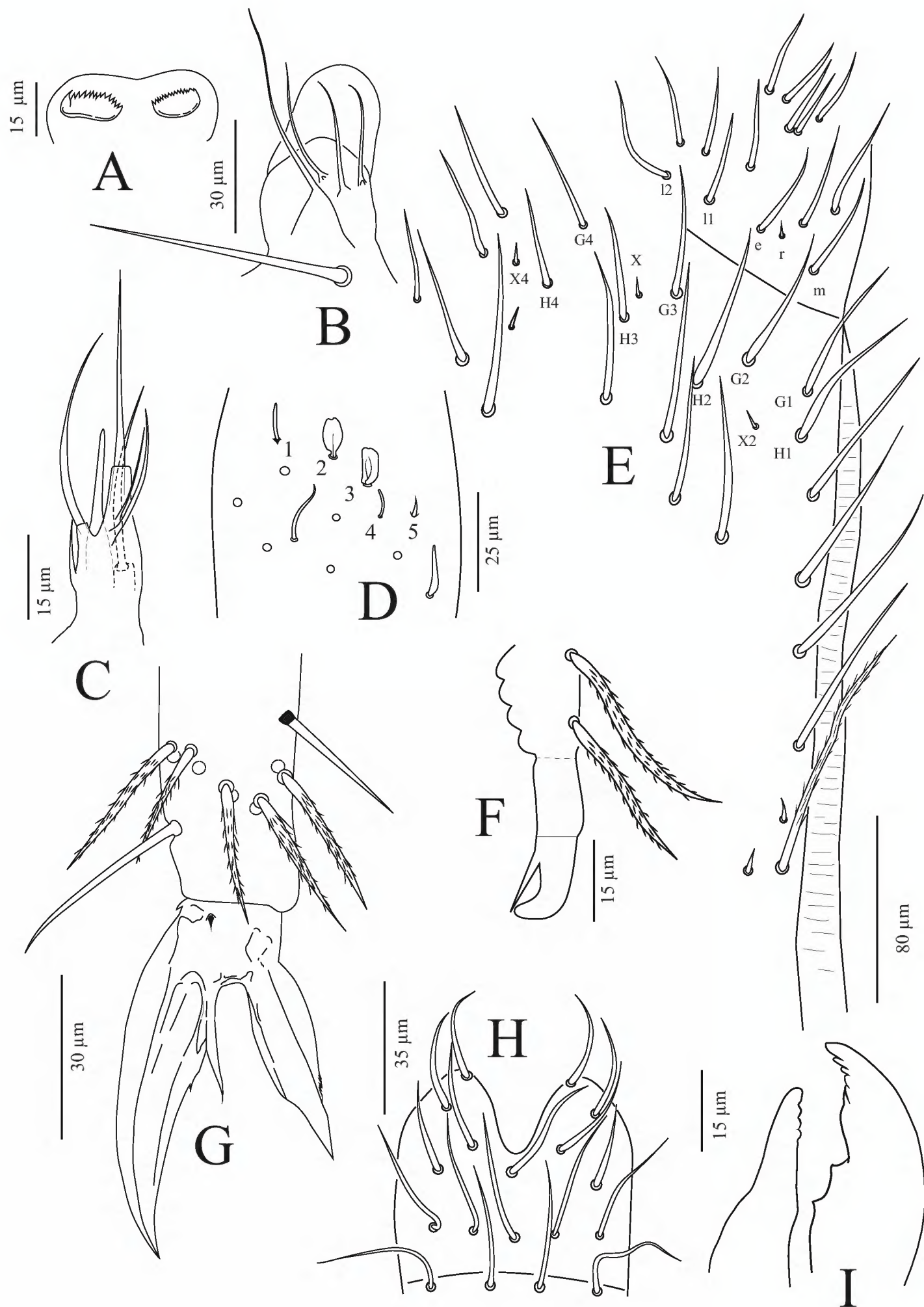
**Description. *Habitus*** (Fig. 1A). Medium size Entomobryidae. Body length 1.8–2.3 mm (holotype 1.8 mm). No scales. Eyes absent. Color: whitish in alcohol, without pigmentation. Four antennal segments. Body slender not bent nor humped at the level of Th. II. Abd. IV 3.80–5.63 times as long as Abd. III along the dorsal midline.

***Pseudopores*** (Figs 3F, 4A–C). Pseudopores present as round flat disks, smaller than mac sockets, except for the coxae and manubrium where psp are as large as mac sockets, present on various parts of the body: antennae, head, tergites, coxae and manubrium. On antennae, psp located ventro-apically between the tip of antennal segments and the chaetae of the apical row, or just below the apical row of chaetae (2 psp on Ant. I, 2–3 psp on Ant. II, and 3 psp on Ant. III). On the head, 1–2 psp located externally on each peri-antennal area. On tergites, 1+1 psp close to the axis from Th. II to Abd. IV (Figs 4A–C). On coxae, 1–2 psp on coxae I, 2–3 psp on coxae II and 1–2 psp on coxae III, located close to longitudinal rows of chaetae. On manubrium, 2+2 dorso-apical ones (Fig. 3F).

***Clypeus and mouthparts*** (Figs 2A–C, 2H, I, 3G). Clypeal area with three long, smooth prefrontal; six middle chaetae (2+2 small ciliated chaetae of unequal size posteriorly and 1+1 rather long, thin, smooth chaetae anteriorly); and 1+1 long smooth lateral chaetae (Fig. 3G). Prelabral and labral chaetae 4/5, 5, 4, all thin and smooth; the three median chaetae of the second row longer than two lateral ones (35–40 vs. 25  $\mu$ m) (Fig. 2H). Distal border of the apical non-granulated area of the labrum with a relatively narrow median U- or V-form intrusion into the granulated area dorsally; apical edge without spines (Fig. 2H). Ventro-distal complex of labrum well differentiated, asymmetrical, with 1+1 distal combs of 14–19 minute teeth on the right side and 14–16 strong and larger teeth on the left side (Fig. 2A), and an axial pair of long sinuous tubules. Maxillary outer lobe with one basal chaeta, one apical chaeta (basal chaeta thicker than apical one) and three smooth sublobal hairs (55–60 vs. 35–38  $\mu$ m) (Fig. 2B). Labial palp strongly modified for the genus, with 0, 5, 0, 4, 4 guards for papillae A–E, as described by Fjellberg (1999) for Entomobryidae or by Xu and Zhang (2015) and Jantarit et al. (2019) for *Coecobrya*. Lateral process of labial palp subcylindrical, as thick as normal chaetae, with tip beyond the apex of the labial papilla (Fig. 2C). Mandible apex blunt and strong, asymmetrical (left with four teeth, right with five teeth) (Fig. 2I); molar plate with three strong pointed basal teeth, and 3–(5) smaller inner distal teeth, identical in both mandibles. Maxilla capitulum with a three-toothed claw and several stout ciliated lamellae; lamella 2 large and broad, lamella 3 well developed; several other lamellae present as figured in Jantarit et al. (2019).

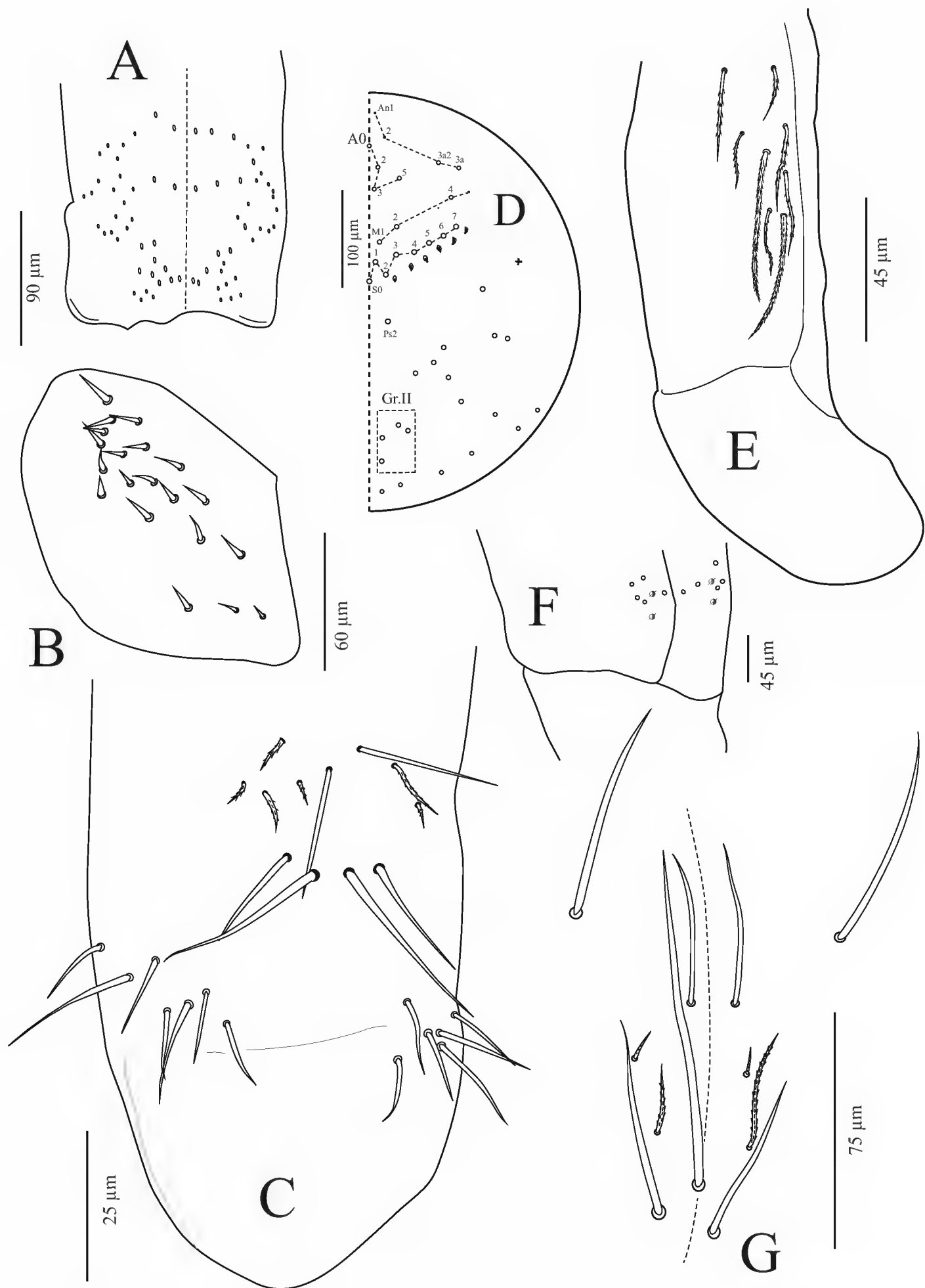
***Antennae*** (Fig. 2D). Antennae very long, approximately 3.78–5.14 times as long as cephalic diagonal. Antennal segments ratio as I: II: III: IV. 1: 2.08–2.20: 2.17–2.21: 3.75–4.40 (N = 3). Antennal segments not subdivided nor annulated. Antennal chaetal types not analysed in detail. Ant. I ventrally with many smooth spiny mic of various sizes in its basal part, many subcylindrical, hyaline sens in its middle to apical part, and many long smooth straight chaetae. The paddle-like chaetae (sensu Nilsai et al. 2017) on Ant II dorsally absent. Ant. III organ with typical five sens, sens one and





**Figure 2.** *Coecobrya whitteni* sp. nov. **A** Ventro-distal complex of labrum **B** Outer maxillary lobe **C** Labial palp **D** Ant. III organ **E** Chaetae of labial basis and ventral chaetotaxy of head **F** Mucro **G** Distal part of tita III and claw complex **H** Prelabral and labral chaetae **I** Mandibles.





**Figure 3.** *Coecobrya whitteni* sp. nov., continued **A** Distal part of manubrium ventrally **B** Trochanteral organ **C** Posterior side of ventral tube and Lateral flap **D** Dorsal cephalic chaetotaxy **E** Anterior side of ventral tube **F** Manubrium plaque **G** Clypeal chaetae.



four subequal, hyaline; sens five acuminate, and shorter; sens two and three swollen in paddle-like chaetae shape (Fig. 2D). Ant. IV without apical bulb. Subapical organite not distinctly knobbed, swollen, slightly enlarged apically, inserted dorsally.

**Dorsal head chaetotaxy** (Fig. 3D). Dorsal cephalic chaetotaxy with four antennal chaetae (An3a2 and An3 as mac, An1–2 as mes), four anterior mac (A0, A2–3 and A5), three median (M1, M2 and M4) and eight sutural (S) mac (S0, S1–7); Gr. II with four mac; A<sub>0</sub> as mac; 5–7+5–7 scale-like structures (usually 6+6) (sensu Jantarit et al. 2019) present below sutural mac, probably inside the integument; a pair of short cephalic trichobothria, external and close to the middle of the head (Fig. 3D).

**Ventral head chaetotaxy** (Fig. 2E). Chaetae of labial basis all smooth (mrel<sub>1</sub>l<sub>2</sub>), chaetae m e and l<sub>1</sub> subequal, r thin and shortest, and l<sub>2</sub> longest. The r/m length ratio: 0.13–0.14 (Fig. 2E). Postlabial chaetae X<sub>2</sub>, X and X<sub>4</sub> smooth, acuminate and minute chaetae, of similar size, X<sub>3</sub> normally absent but present as mic in one individual. On each side of the cephalic groove with 9 chaetae, of which, the proximal six always long and smooth, 7<sup>th</sup> and 9<sup>th</sup> as mics, 8<sup>th</sup> always long and ciliated, one specimen with three mics distally (Fig. 2E).

**Tergite chaetotaxy** (Figs. 4A–D). Th. II with two (m1, m2) medio-medial, one (m4) medio-sublateral and 18–20 posterior mac; 1+1 ms and 2+2 sens antero-laterally (Fig. 4A).

Th. III with 23–26 mac. 2+2 sens laterally (Fig. 4A).

Abd. I with six central mac (m4i, m2–4, m2i, m4p), 1+1 ms and 1+1 sens laterally (Fig. 4B).

Abd. II with two (m3, m3e) central and one (m5) lateral mac, 2+2 tric without modified chaetae, 1+1 sens laterally and 1+1 mic near internal tric (Fig. 4B).

Abd. III with one (m3) central and three (am6, pm6, p6) lateral mac. 3+3 tric without modified chaetae, 1+1 sens laterally, 1+1 mic near m3, ms not seen (Fig. 4B).

Abd. IV with four central mac (M, A5–6, B5) and six (D3, E2–4, E2p, F3) lateral mac, 2+2 tric and about 19 long S-like chaetae and one mic in the middle below psp, without modified chaetae (Fig. 4C).

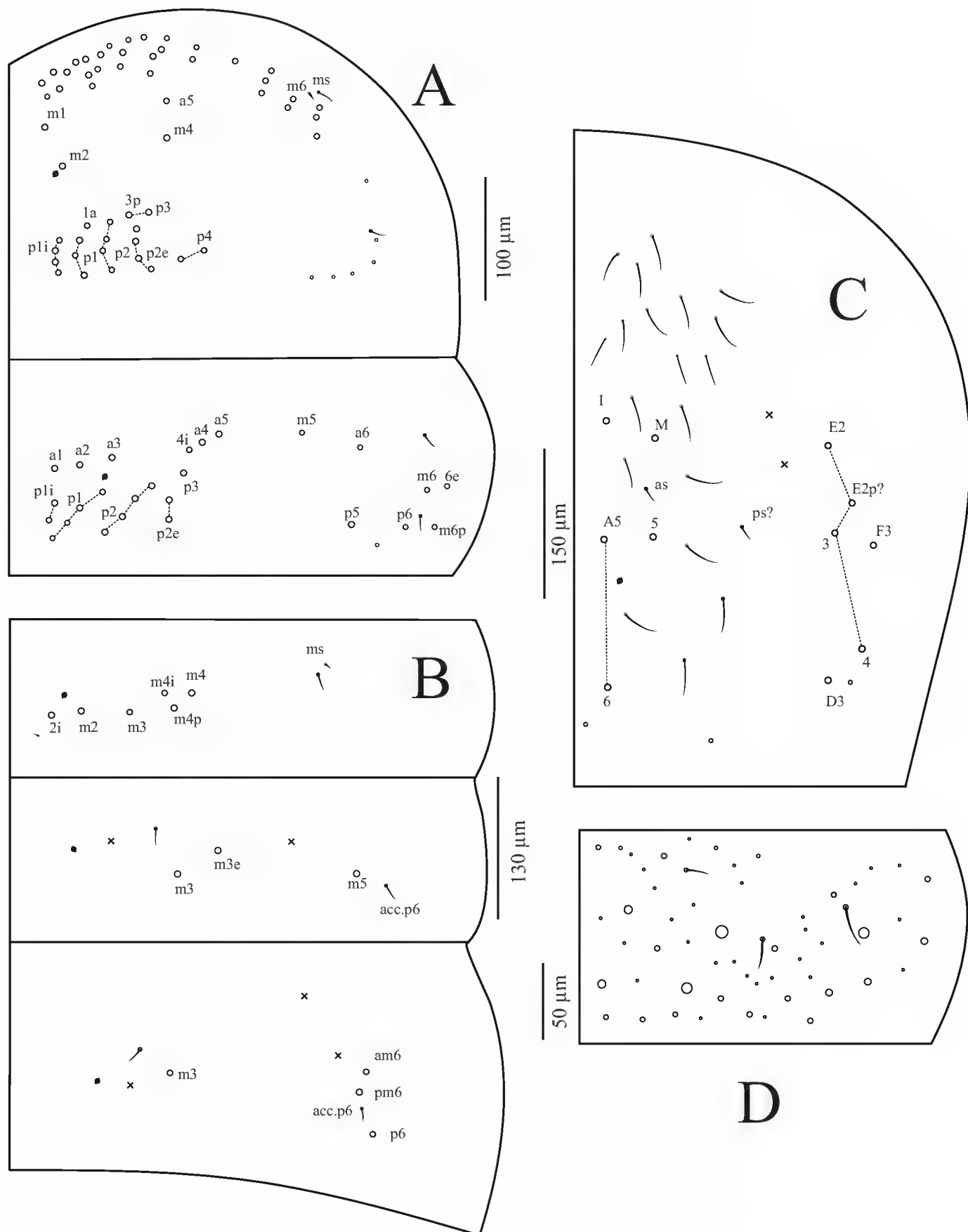
Abd. V with at least 6 obvious mac and several mes to small mac, and 3+3 sens (Fig. 4D).

Abd. VI not analysed.

S-chaetae formula from Th. II to Abd. V: 2+ms, 2/1+ms, 2, 2, ≈ 20, 3; ps not seen, as sens on Abd. IV 1/3 as long as S-like chaetae (Figs 4A–D).

**Legs** (Figs 2G, 3B). Leg long; tita of leg III slightly longer than tita of legs I and II. Legs devoid of scales, covered with ordinary ciliated chaetae of various lengths, mic not seen. Trochanteral organ with 18–21 smooth, straight, unequal spine-like chaetae (Fig. 3B). The distal whorl of tita III with 10 subequal ciliated mes, irregularly arranged, and usually claw I–II with dorso-apical clavate tenent hair (pointed in two specimens). A smooth, thin and long chaeta close to tenent hair (sensu Jantarit et al. 2019) absent. Claw III generally with dorso-apical clavate tenent hair (8 individuals with clavate tenent hair, 4 individuals with pointed tenent hair; holotype with pointed tenent hair Fig. 2G). Ventro-distal smooth chaeta of tita III thick, erected, pointed, rather short. Pretarsal mic minute (2.5–3.0 μm). Claw slender and elongated. Unguis of all claw





**Figure 4.** *Coecobrya whitteni* sp. nov., continued **A** Chaetotaxy of dorsal Th. II– III **B** Chaetotaxy of dorsal Abd. I– III **C** Chaetotaxy of dorsal Abd. IV **D** Chaetotaxy of dorsal Abd. V.

with one small inner teeth at 53 % and a pair of unequal basal teeth at about 44–49 % of inner edge from basis. Unguiculus approximately 2/3 as long as inner edge of claw, rather swollen baso-externally, pointed apically, devoid of inner tooth, with at least five minute outer teeth, often inconspicuous, at 3/4 of its length (Fig. 2G).



**Ventral tube** (Figs 3C, E). Ventral tube two to three times longer than wide. Lateral flaps with 6–7+6–7 smooth chaetae except for one specimen with one ciliated chaeta present in both sides (Fig. 3C). Anterior face with 6–8+6–8 chaetae, 2(3) of them larger than others, all ciliated, arranged roughly asymmetrically (Fig. 3E); posterior face with at least 12 chaetae, four apical chaetae longer and larger than others and about 8–13 proximal chaetae; two straight smooth chaetae and 6–11 either small finely ciliated or mics arranged asymmetrically (Fig. 3C).

**Furcal complex** (Figs 2F, 3A, F). Tenaculum with four large teeth of decreasing size from the basal to the distal one of each ramus, on a prominent, irregular body, with a postero-basal strong serrated chaeta bent distally. Mucrodens 1.6 times longer than manubrium. Furcula without smooth chaetae. Manubrium with a dense cover of ciliated chaetae both dorsally and ventrally. Manubrial plaque with 2+2 psp and three to six ciliated chaetae (usually 5+5) (Fig. 3F). Distal part of manubrium ventrally with 13+13 ciliate chaetae (Fig. 3A). Dens without spines, annulated and covered with ciliated chaetae on both sides. Distal smooth part of dens slightly longer than mucro. Mucro strong and falcate, basal spine long, reaching the tip of the mucronal tooth (Fig. 2F).

**Genital plate.** Female genital plate with 2+2 genital mic, male genital plate not clearly seen.

**Ecology.** *Coecobrya whitteni* sp. nov. was found in the dark zone of a cave on the surface of the muddy ground, near the bank of a stream. It was also found in the upper levels on stalagmites, clay, gravel and rock surfaces. This species was found mainly in eutrophic habitats where piles of guano were well-presented. There was a big colony of the intermediate roundleaf bat (*Hipposideros larvatus*) inside the cave. The air temperature of the habitat where this species was collected was 22.9–24.0 °C and the relative humidity was 85%. In the same cave, and in the same chamber, at least six different stygobitic species were also observed in the small puddles: *Aequigidiella aequilifera*, *Dugesia deharvengi*, *Heterochaetella glandularis*, *Siamoporus deharvengi*, *Stenansellus rigali* and *Theosbaena cambodjiana*. The co-occurrence of these six species was first observed in 1987 (Association Pyrénéenne de Spéléologie 1988; Deharveng and Bedos 2000, 2012) and from our observations they are all still present in rather large numbers of individuals. We also found a millipede (*Plusioglyphiulus saksit*) and a spider (*Speocera deharvengi*) in the same habitat where this new species was found. Most of these observed species only known as endemics to this cave. The discovery of this new species in Tham Nayn Noi emphasizes the importance of this cave in harboring the endemic subterranean fauna of the area.

**Etymology.** *Coecobrya whitteni* sp. nov. is named in honor of the late Tony Whitten in appreciation of his enormous contributions to the discovery and conservation of karst and cave invertebrates across Asia.

**Remarks.** Among the described troglomorphic *Coecobrya* species in Thailand, *Coecobrya whitteni* sp. nov. is near to *C. cavicta* Nilsai & Zhang, 2017 from Satun province, southern Thailand in the body length, dorsal head chaetotaxy, labial chaetae, number of sublobal hairs on maxillary outer lobe, number of mac on Abd.III and number of chaetae on ventrodiscal part of manubrium. However, it differs from



*C. cavicta* by the combination of various characters i.e. longer length of antennae, number of mac of dorsal tergites on Th. II (21–23 vs. 36–37), Th. III (23–26 vs. 35), Abd. II (2 vs. 3) and Abd. IV (11 vs. 18–20), number of inner teeth of claw (3 vs. 2) and number of smooth chaetae of trochanteral organe (18–21 vs. 15–16) (see Table 1). In fact, *Coecobrya whitteni* sp. nov. is most similar to *C. troglobia* sp. nov. which is described in this work since the caves where these two species are found are only 3.4 km apart in a straight line, separated by limestone mountains. The altitudes of the two caves differs by about 200 m. The two species are similar in body size, antennal length, number of sublobal hair on the maxillary outer lobe, number of chaetae on Gr. II, labial chaetae, number of mac on Abd. I–IV, number of inner teeth of the claw, tenent hair, similar number in ventral tube chaetae both anterior and posterior side as well as a lateral flap, number of chaetae on trochanteral organ (Table 1). However, *Coecobrya whitteni* sp. nov. differs from *C. troglobia* sp. nov. by the number of An dorsal mac on the head (4 vs. 5) and number of chaetae on Th. II (medio-medial mac = 2 vs. 3, medio-sublateral mac = 1 vs. 4) (Table 1). *Coecobrya whitteni* sp. nov. has in fact already been mentioned by Deharveng and Bedos (2000) as an undescribed cf. *Coecobrya* species (Fig. 31.8A page 625) from oligotrophic habitats. (Note that in this reference the cave is misnamed as Tham Kubio).

***Coecobrya troglobia* Jantarit & Nilsai, sp. nov.**

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Figures 1B, 5–7, Table 1

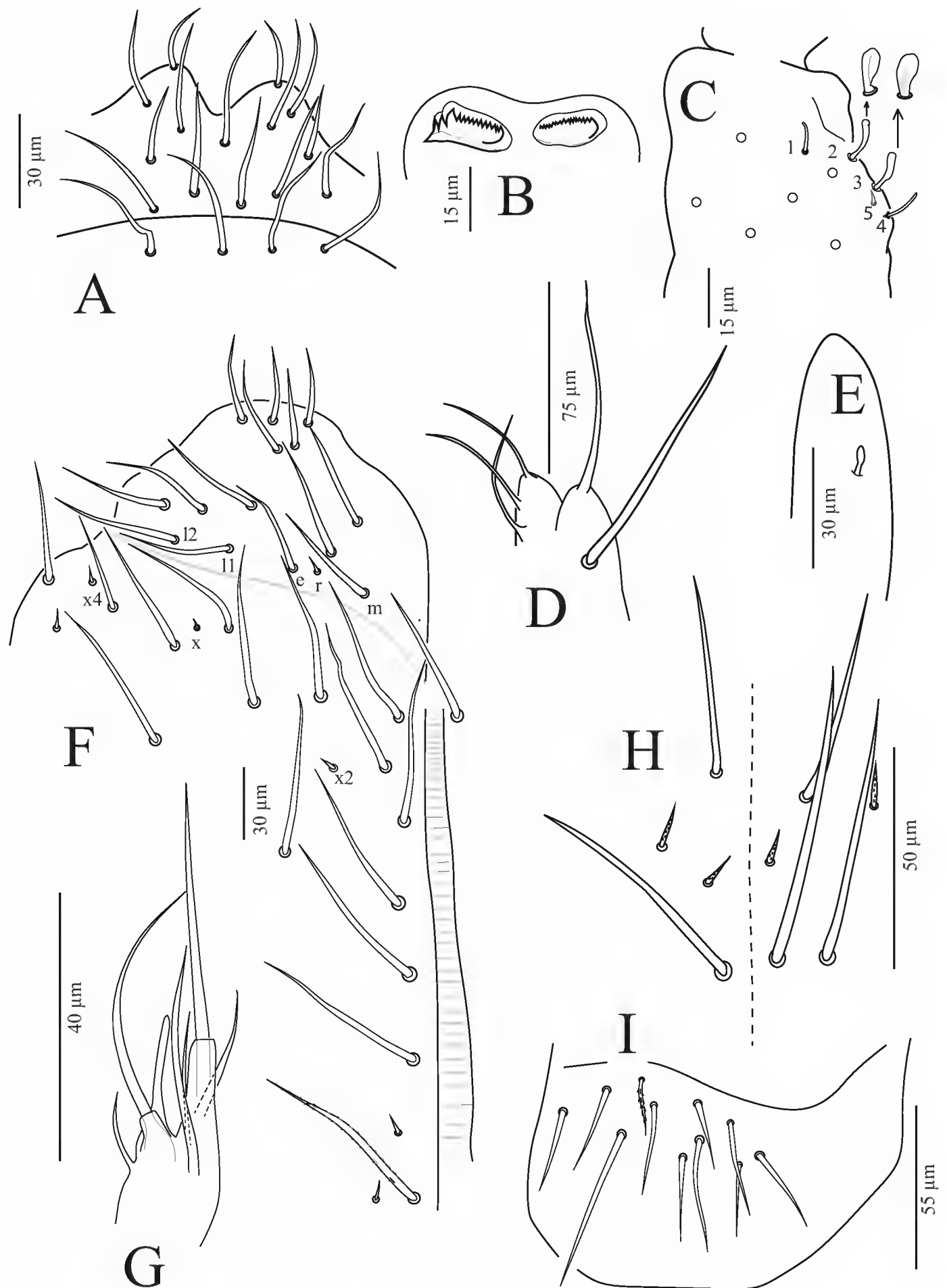
**Type material. Holotype:** female on slide. Thailand, Khon Kaen province, Chum Phae district, Tham Phaya Nakharat, altitude 562 m a.m.s.l., 16°48'50.5"N, 101°57'23.9"E. 3.XII.2020; S. Jantarit, A. Nilsai, K. Sarakhamhaeng and K. Jantapaso leg. (sample # THA\_SJ\_KKN03), dark zone of a cave, by entomological aspirator. **Paratypes:** same data as holotype, 7 specimens (3 females and 4 subadults on slides). **Additional material:** same data as holotype, 6 specimens (in ethanol).

Holotype and seven paratypes on slides deposited in NHM-PSU.

**Description. Habitus** (Fig. 1B). Medium size Entomobryidae. Body length 2.0–2.4 mm (holotype 2.4 mm). No scales. Eyes absent. Color: whitish in alcohol, without pigmentation. Four antennal segments (sometimes Ant. II and III fused together). Body slender not bent nor humped at the level of Th. II., elongated antennae, legs and furca. Th. II slightly larger than Th. III; Abd. IV about 3.5 times as long as Abd. III along the dorsal midline.

**Pseudopores** (Figs 6D, 7A–C). Pseudopores present as round flat disks, smaller than mac sockets (Figs 7A–C), except for the coxae and manubrium where psp are as large as mac sockets, present on various parts of the body: antennae, head, tergites, coxae and manubrium. On antennae, psp located ventro-apically between the tip of antennal segments and the chaetae of the apical row, or just below apical row of chaetae (2 psp on Ant. I, 2–3 psp on Ant. II, and 3 psp on Ant. III). On head, 1–2





**Figure 5.** *Coecobrya troglobia* sp. nov. **A** Prelabral and labral chaetae **B** Ventro-distal complex of labrum **C** Ant. III organ **D** Outer maxillary lobe **E** Subapical organ of Ant. IV **F** Chaetae of labial basis and ventral chaetotaxy of head **G** Labial palp **H** Clypeal chaetae **I** Lateral flap.

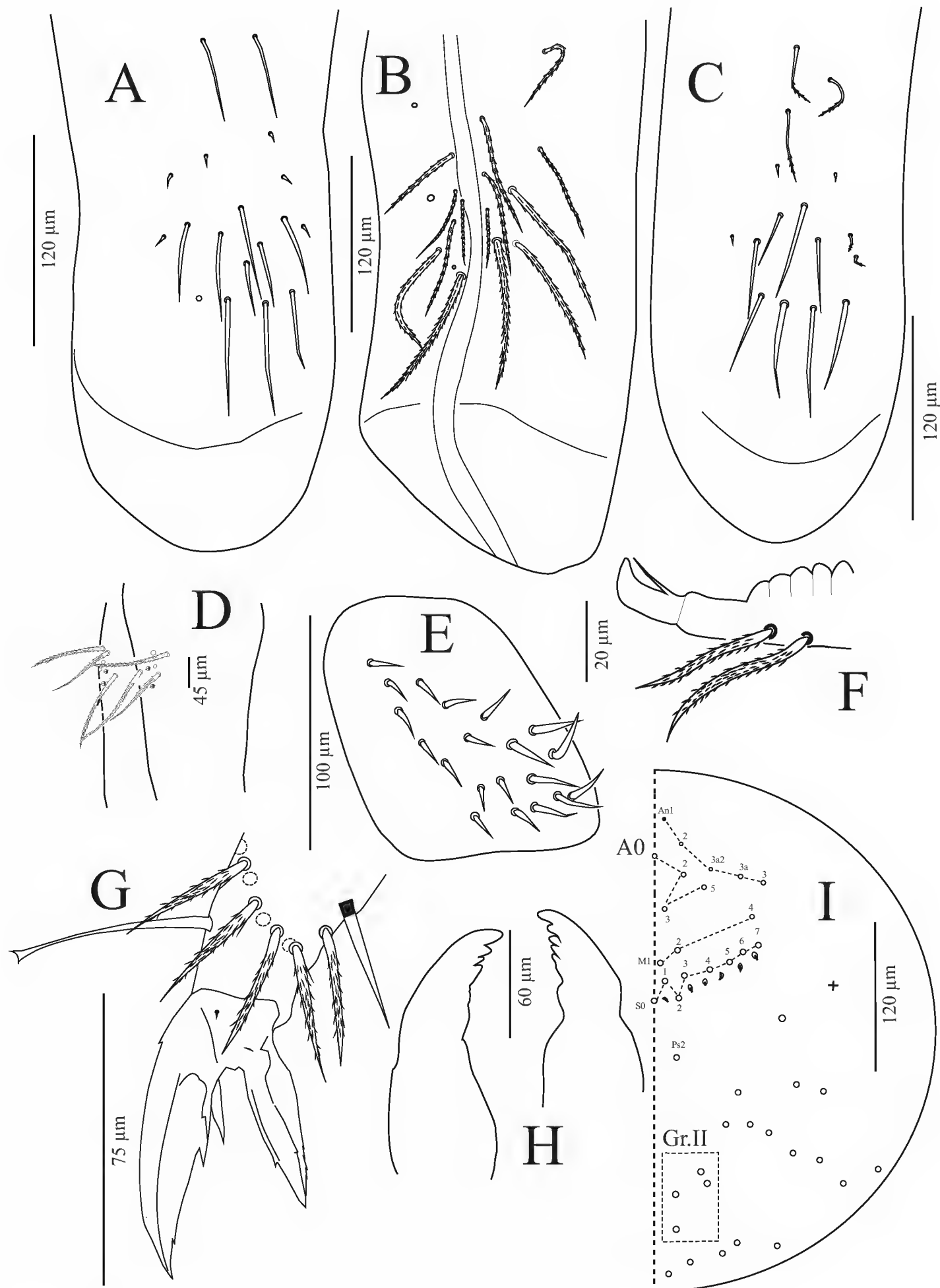
psp located externally on each peri-antennal area. On tergites, 1+1 psp close to the axis from Th. II to Abd. IV (Figs 7A–C). On coxae, 1–2 psp on coxae I, 2–3 psp on coxae II and 1–2 psp on coxae III, located close to longitudinal rows of chaetae. On manubrium, 2+2 dorso-apical ones (Fig. 6D).

**Clypeus and mouthparts** (Figs 5A, B, D, G, H, 6H). Clypeal area with nine chaetae arranged in three rows, three long smooth prefrontal, 2+2 small ciliated chaetae sometimes asymmetric arrangement, and 1+1 long smooth facial chaetae, the lateral long smooth chaetae not seen (Fig. 5H). Prelabral and labral chaetae 4/5, 5, 4, all thin and smooth; three median chaetae of the second rows longer and slightly larger than those of the distal and proximal rows and longer than lateral ones (35–40 vs. 25–30  $\mu\text{m}$ ) (Fig. 5A). Distal border of the apical non-granulated area of the labrum with a relatively narrow median U- or V-form intrusion into the granulated area dorsally; apical edge without spines (Fig. 5A). Ventro-distal complex of labrum well differentiated, asymmetrical, with 1+1 distal combs of 14–16 minute on the right side and 14–16 strong and larger teeth on the left side (Fig. 5B), and an axial pair of long sinuous tubules. Maxillary outer lobe with one basal chaeta, one apical chaeta (basal chaeta thicker than apical one) and three smooth sublobal hairs (60–65 vs. 35–38  $\mu\text{m}$ ) (Fig. 5D). Labial palp strongly modified for the genus, with 0, 5, 0, 4, 4 guards for papillae A–E. Lateral process of labial palp subcylindrical, as thick as normal chaetae with tip beyond the apex of the labial papilla (Fig. 5G). Mandible apex strong, asymmetrical (left with four teeth, right with five teeth) (Fig. 6H); molar plate with three strong pointed basal teeth, and 3–(5) smaller inner distal teeth, identical in both mandibles. Maxilla capitulum with a three-toothed claw and several stout ciliated lamellae; lamella 2 large and broad, lamella 3 well developed; several other lamellae present.

**Antennae** (Figs 5C, E). Antennae very long, approximately 4.0–6.8 times as long as cephalic diagonal. Antennal segments ratio as I: II: III: IV = 1: 1.1–2.7: 0.7–2.4: 2.1–4.6 (N = 5). Antennal segments not subdivided nor annulated. Antennal chaetal types not analysed in detail. Smooth spiny mic at base of antennae: 3 dorsal, 3 ventral on Ant. I, 1 internal, 1 external and 1 ventral on Ant. II, 1 smooth ventral on Ant. I, smooth straight long chaetae on antennae present. Ant. I ventrally with many smooth spiny mic of various sizes in its basal part, many subcylindrical, hyaline sens in its middle to apical part, and many long smooth straight chaetae. The paddle-like chaetae on Ant II absent. Ant. III organ with five sens; sens one and four subequal, hyaline; sens five acuminate, dark and shorter; sens two and three swollen (Fig. 5C). Ant. IV very long, not subdivided, without apical bulb. Subapical organite not distinctly knobbed, swollen, slightly enlarged apically, inserted dorsally (Fig. 5E).

**Dorsal head chaetotaxy** (Fig. 6I). Dorsal cephalic chaetotaxy with five antennal chaetae (An1–3, An3a, An3a2, An1 as mes), four anterior mac (A0, A2–3 and A5) three median (M1–2, M4) and eight sutural mac (S0, S1–7); Gr. II with four mac; A<sub>0</sub> as mac; 6+6 scale-like structures present below sutural mac, probably inside the integu-





**Figure 6.** *Coecobrya troglobia* sp. nov., continued **A** Posterior side of ventral tube **B** Anterior side of ventral tube **C** Posterior side of ventral tube **D** Distal part of manubrium ventrally **E** Trochanteral organ **F** Mucro **G** Distal part of tita III and claw complex **H** Mandibles **I** Dorsal cephalic chaetotaxy.

ment; a pair of short cephalic trichobothria, external and close to the middle of the head (Fig. 6I).

**Ventral head chaetotaxy** (Fig. 5F). Chaetae of labial basis all smooth ( $mrel_1l_2$ ), chaetae  $m$ ,  $e$  and  $l_1$  subequal,  $r$  thin and shortest, and  $l_2$  longest, The  $r/m$  length ratio: 0.11–0.16 (Fig. 5F). Postlabial chaetae  $X_2$ ,  $X$  and  $X_4$  smooth and minute chaetae,  $X_1$  and  $X_3$  absent. On each side of cephalic groove with 9 chaetae, of which the anterior six always long and smooth, 7<sup>th</sup> and 9<sup>th</sup> as mics, 8<sup>th</sup> always long and ciliated, one specimen with three mics distally in one side (Fig. 5F).

**Tergite chaetotaxy** (Figs 7A–D). Th. II with three ( $m1$ ,  $m2$ ,  $m2i2$ ) medio-medial, two ( $m4$ ,  $m4p$ ) medio-sublateral and 15–18 posterior mac; 1+1  $ms$  and 2+2  $sens$  antero-laterally (Fig. 7A).

Th. III with 24–27 mac,  $a4i$  and  $a6i$  as mac, 2+2  $sens$  laterally (Fig. 7A).

Abd. I with six ( $m2-4$ ,  $m2i$ ,  $m4p$ ,  $m4i$ ) mac, 1+1  $ms$  and 1+1  $sens$  laterally (Fig. 7B).

Abd. II with two ( $m3$ ,  $m3e$ ) central and one ( $m5$ ) lateral mac. 2+2 tric without modified chaetae, 1+1  $sens$  laterally and 1+1 mic near internal tric (Fig. 7B).

Abd. III with one ( $m3$ ) central, and three ( $am6$ ,  $pm6$ ,  $p6$ ) lateral mac. 3+3 tric without modified chaetae, 1+1  $sens$  laterally, 1+1 mic near  $m3$ ,  $ms$  not seen (Fig. 7B).

Abd. IV with five central mac ( $I$ ,  $A5-6$ ,  $B4-5$ ) and six ( $D3$ ,  $E1$ ,  $E2p$ ,  $E3-4$ ,  $F3$ ) lateral mac, 2+2 tric and about 18 long S-like chaetae anteriorly, without modified chaetae (Fig. 7C).

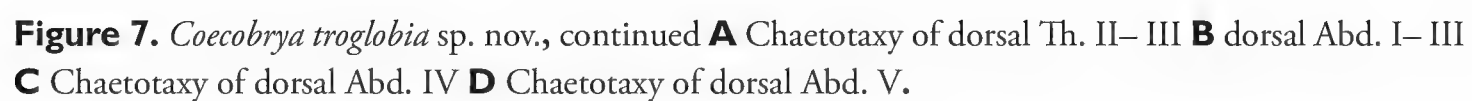
Abd. V with 15–18 mac and several  $mes$  to small mac, and 3+3  $sens$  (Fig. 7D).

Abd. VI not analysed. S-chaetae formula from Th. II to Abd. V: 2+ $ms$ , 2/1+ $ms$ , 2, 2,  $\approx 15-18$ , 3;  $ps$  not seen, as  $sens$  on Abd. IV 1/3 as long as S-like chaetae (Figs 7A–D).

**Legs** (Figs 6E, G). Leg long; tita of leg III slightly longer than tita of legs I and II. Legs devoid of scales, covered with ordinary ciliated chaetae of various lengths, mic not seen. Trochanteral organ with 19–23 smooth, straight, unequal spine-like chaetae (Fig. 6E). The distal whorl of tita with 9–10 subequal ciliated  $mes$ , irregularly arranged, and usually dorso-apical clavate tenent hair present (two specimens claw III with pointed tenent hair). A smooth, thin and long chaeta close to the absent tenent hair. Ventro-distal smooth chaeta of tita III thick, erected, pointed, rather short. Claw rather slender and elongated. Unguis of all claws with two inner teeth, one-minute tooth at about 90 % from base of the claw, and a strong inner tooth at 40–46 %, and a pair of subequal basal teeth at about 30–34 % of inner edge from basis. Unguiculus approximately 2/3 as long as inner edge of the claw, rather swollen baso-externally, pointed apically, with one inner tooth and at least four minute outer teeth, often inconspicuous near the tip of its length (Fig. 6G).

**Ventral tube** (Figs 5I, 6A–C). Ventral tube three to four times longer than wide. Lateral flaps usually with 7–9+7–9 smooth chaetae, sometimes 1–2 ciliated present. In the holotype, 10 smooth and one ciliated chaeta present on one side (Fig. 5I). Anterior face with 7–9+7–9 ciliated chaetae, three of them apically larger than others (Fig. 6B); posterior face with 4 long, smooth chaetae apically, the middle chaetae





with 4–6 long, smooth chaetae mixed with 5–6 mic arranged roughly asymmetrically, and 2–3 proximal chaetae, ciliated in the holotype (Fig. 6C) but smooth in 2 specimens (Fig. 6A).

**Furcal complex** (Figs 6D, F). Tenaculum with four large teeth of decreasing size from the basal to the distal one of each ramus, on a prominent, irregular body, with a postero-basal strong serrated chaeta bent distally. Mucrodens 1.15–1.42 times longer than manubrium. Furcula without smooth chaetae. Manubrium densely covered of ciliated chaetae both dorsally and ventrally. Manubrial plaque with 2+2 psp and five ciliated chaetae (Fig. 6D). Distal part of the ventral manubrium not clearly seen in all specimens. Dens without spines, annulated and covered with ciliated chaetae on both sides. Distal smooth part of dens as long as the mucro. Mucro strong and falcate, basal spine long, nearly reaching the tip of the mucronal tooth (Fig. 6F).

**Genital plate.** Female genital plate with 2+2 genital mic.

**Ecology.** *Coecobrya troglobia* sp. nov. is restricted to the dark zone of the cave, where it was found in two chambers in an oligotrophic environment. Most individuals were found on the floor, on stalagmites and on the rock walls. The temperature in the dark zone was 21.4–23 °C and the relative humidity was about 75 %.

**Etymology.** The name of the new species is derived from the Greek stem “trogle (τρώγλη)” which means “hole”, referring to the habitat of this new species which is restricted to the subterranean environment.

**Remarks.** *Coecobrya troglobia* sp. nov. is also near to *C. cavicta* Nilsai & Zhang, 2017 from Satun province, southern Thailand in the number of mac of M series and Gr. II of dorsal head chaetotaxy, labial chaetae, number of sublobal hairs on maxillary outer lobe, number of mac on medio-medial area of Th.II, number of mac on Abd. III. However, *Coecobrya troglobia* sp. nov. is different from *C. cavicta* by having bigger body size, longer length of antennae, number of chaetae along cephalic groove, number of mac of dorsal tergites on Th. II (20–23 vs. 36–37), Th. III (24–27 vs. 35), Abd. II (2 vs. 3) and Abd. IV (11 vs. 18–20), number of inner teeth of claw (3 vs. 2) and number of smooth chaetae of trochanteral organ (19–23 vs. 15–16) (see Table 1). *Coecobrya troglobia* sp. nov. is close to *C. whitteni* sp. nov. from Tham Nayn Noi in Chum Phae district, Khon Kaen province, but they clearly differ from each other in a few morphological characters, see the detailed diagnosis in the remarks under *C. whitteni* sp. nov. and Table 1.

***Coecobrya ellisi* Jantarit & Nilsai, sp. nov.**

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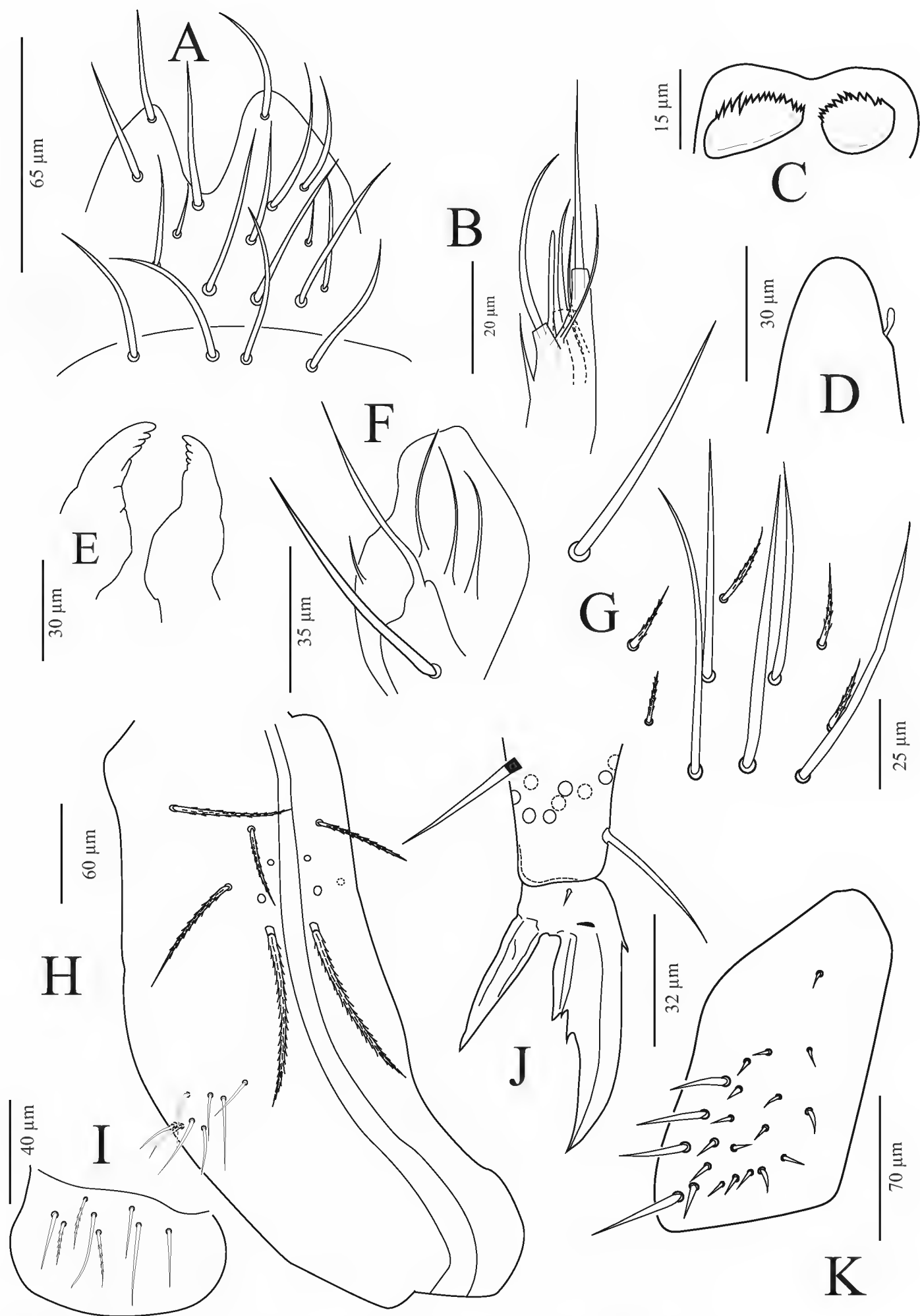
Figures 1C, 8–10, Table 1

**Type material. Holotype:** male on slide. Thailand, Phetchabun province, Nong Phai district, Tham Tho, altitude 261 m a.m.s.l., 16.1231°N, 101.0222°E. 9.X.2019; S. Jantarit and A. Nilsai leg. (sample # THA\_SJ\_PNB06), dark zone of a cave, by entomological aspirator. **Paratypes:** same data as holotype, 12 specimens (1 male, 4 females and 7 subadults on slides). **Additional material:** same data as holotype, 8 specimens (in ethanol).

Holotype and 12 paratypes on slides deposited in NHM-PSU.

**Description. Habitus** (Fig. 1C). Medium size Entomobryidae. Body length 1.6–2.1 mm (holotype 1.8 mm). No scales. Eyes absent. Color: whitish in alcohol, with





**Figure 8.** *Coecobrya ellisi* sp. nov. **A** Prelabral and labral chaetae **B** Labial palp **C** Ventro-distal complex of labrum **D** Subapical organ of Ant. IV **E** Mandibles **F** Outer maxillary lobe **G** Clypeal chaetae **H** Anterior side of ventral tube and Lateral flap **I** Lateral flap **J** Distal part of tita III and claw complex **K** Trochanteral organ.

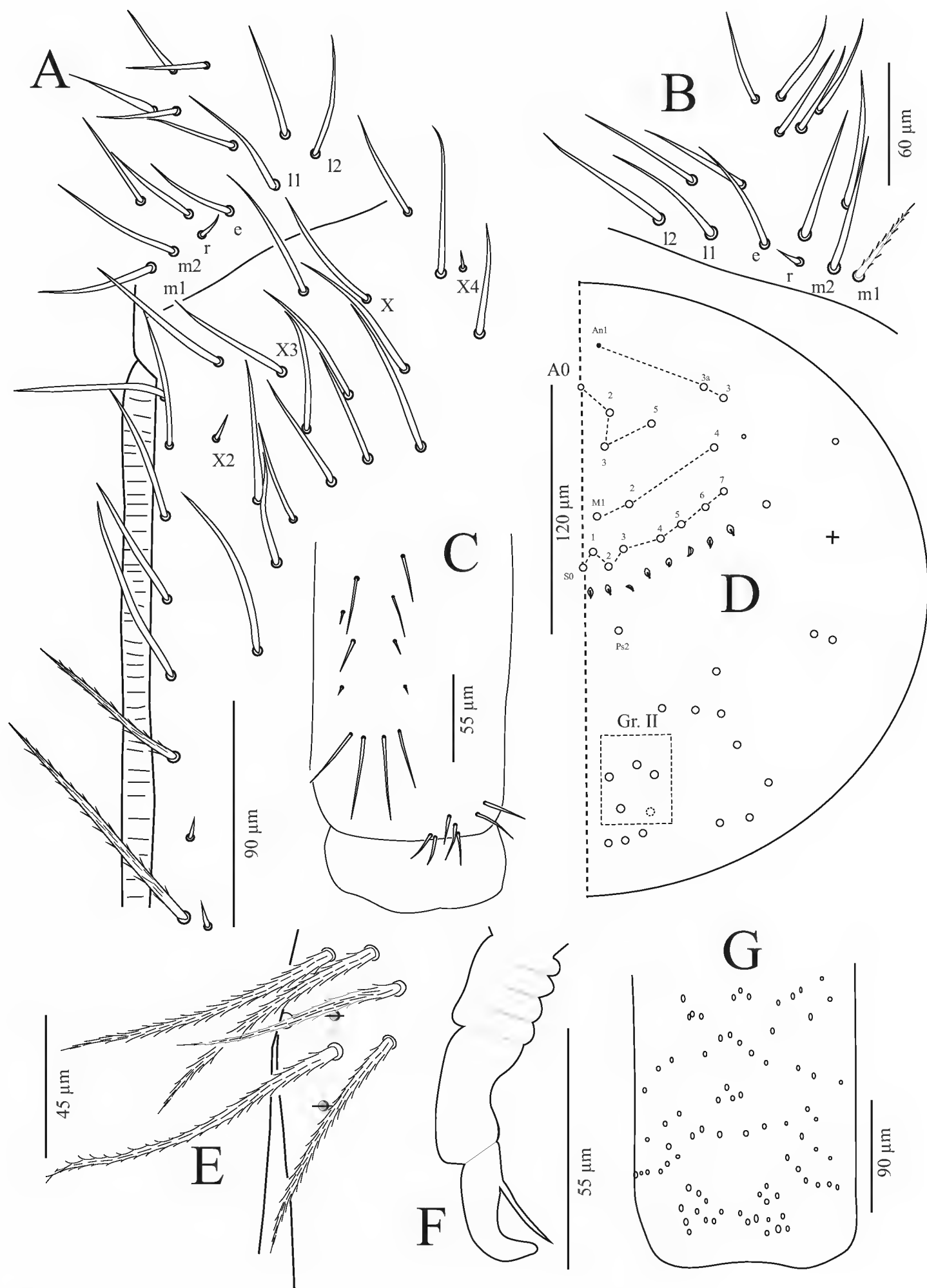
orange pigmentation on head, body, legs and furca. Four antennal segments. Body slender not bent nor humped at the level of Th. II. Th. II slightly larger than Th. III. Abd. IV 3.70–4.25 times as long as Abd. III along dorsal midline.

**Pseudopores** (Figs 9E, 10A–C). Pseudopores present as round flat disks, smaller than mac sockets, except for the coxae and manubrium where psp are as large as mac sockets, present on various parts of the body: antennae, head, tergites, coxae and manubrium. On antennae, psp located ventro-apically between the tip of antennal segments and the chaetae of the apical row, or just below apical row of chaetae (2 psp on Ant. I, 2–3 psp on Ant. II, and 3 psp on Ant. III). On head, 1–2 psp located externally on each peri-antennal area. On tergites, 1+1 psp close to the axis from Th. II to Abd. IV (Figs 10A–C). On coxae, 1–2 psp on coxae I, 2–3 psp on coxae II and 1–2 psp on coxae III, located close to longitudinal rows of chaetae. On manubrium, 2+2 dorso-apical ones (Fig. 9E).

**Clypeus and mouthparts** (Figs 8A–C, E–G). Clypeal area with three long, smooth prefrontal; seven middle chaetae (two long smooth chaetae and five ciliated chaetae; 1+1 long lateral chaetae (Fig. 8G). Prelabral and labral chaetae 4/5, 5, 4, all thin and smooth; the three median chaetae of the first row longer than two lateral ones, two lateral chaetae 1/2.5 length of others on second row (Fig. 8A). Distal border of the apical non-granulated area of the labrum with a relatively narrow median U- or V-form intrusion into the granulated area dorsally; apical edge without spines (Fig. 8A). Vento-distal complex of labrum well differentiated, asymmetrical, with 1+1 distal combs of 13–15 minute on the right side and 14–16 strong and larger teeth on the left side (Fig. 8C), and an axial pair of long sinuous tubules. Maxillary outer lobe with one basal chaeta, one apical chaeta (basal chaeta thicker than apical one) (34–44  $\mu\text{m}$  vs. 12–15  $\mu\text{m}$ ) and four smooth sublobal hairs (Fig. 8F). Labial palp strongly modified for the genus, with 0, 5, 0, 4, 4 guards for papillae A–E. Lateral process of labial palp subcylindrical, as thick as normal chaetae, with tip beyond the apex of the labial papilla (Fig. 8B). Mandible apex strong, asymmetrical (left with four teeth, right with five teeth); molar plate with three strong pointed basal teeth, and 3–(5) smaller inner distal teeth, identical in both mandibles (Fig. 8E). Maxilla capitulum with a three-toothed claw and several stout ciliated lamellae; lamella 2 large and broad, lamella 3 well developed; several other lamellae present.

**Antennae** (Fig. 8D). Antennae extremely long, approximately 3.5–6.4 times as long as cephalic diagonal. Antennal segments ratio as I: II: III: IV. 1: 1.85: 1.76: 2.54 (N = 3). However, Ant II and III fused together in most cases. Antennal segments not subdivided nor annulated. Antennal chaetal types not analysed in detail. Smooth spiny mic at base of antennae: 3 dorsal, 3 ventral on Ant. I, 1 internal, 1 external and 1 ventral on Ant. II, 1 smooth ventral on Ant. I, smooth straight long chaetae on antennae present Ant. I ventrally with many smooth spiny mic of various sizes in its basal part, many subcylindrical, hyaline sens in its middle to apical part, and many long smooth straight chaetae. The paddle-like chaetae on Ant II absent. Ant. III organ with five sens not clearly seen in all specimens. Ant. IV without apical bulb. Subapical organite not distinctly knobbed, swollen, slightly enlarged apically, inserted dorsally (Fig. 8D).





**Figure 9.** *Coecobrya ellisi* sp. nov., continued **A** Chaetae of labial basis and ventral chaetotaxy of head **B** Chaetae of labial basis **C** Posterior side of ventral tube and Lateral flap **D** Dorsal cephalic chaetotaxy **E** Manubrium plaque **F** Mucro **G** Distal part of manubrium ventrally.

**Dorsal head chaetotaxy** (Fig. 9D). Dorsal cephalic chaetotaxy with three antennal chaetae (An1, An3a, An3, An1 as mes); four anterior mac (A0, A2–3 and A5), three median (M1, M2 and M4) and eight sutural mac (S0, S1–7); Gr. II with 4(5) mac; A<sub>0</sub> as mac; 6–8+6–8 scale-like structures (usually 6+6) present below sutural mac, probably inside the integument; a pair of short cephalic trichobothria, external and close to the middle of the head (Fig. 9D).

**Ventral head chaetotaxy** (Figs 9A, B). Chaetae of labial basis almost all smooth ( $m_1m_2rel_1l_2$ ) or ( $m_1mrel_1l_2$  sensu Zhang and Pan, 2020) (Fig. 9A) except  $m_1(mi)$  ciliated in one side in two specimens (Fig. 9B), chaetae  $m_1$ ,  $e$  and  $l_1$  subequal,  $r$  thin and shortest, and  $l_2$  longest,  $m_2$  longer and thicker than  $m_1$ . The  $r/m_2$  length ratio: 0.15–0.21 (Fig. 9A). Postlabial chaetae  $X_2$ , and  $X_4$  smooth and minute chaetae,  $X$ ,  $X_3$  long and smooth and  $X_1$  absent (Fig. 9A). On each side of the cephalic groove with 8–9 chaetae, of which the anterior five always long and smooth, 6<sup>th</sup> and 8<sup>th</sup> long and ciliated, 7<sup>th</sup> and 9<sup>th</sup> mics (Fig. 9A).

**Tergite chaetotaxy** (Figs 10A–D). Th. II with three ( $m_1$ ,  $m_2$ ,  $m_{2i}$ ) medio-medial, three ( $m_4$ ,  $m_{4p}$ ,  $m_{4i}$ ) medio-sublateral and 28–31 posterior mac; 1+1 ms and 2+2 sens antero-laterally (Fig. 10A).

Th. III with 29–36 mac. 2+2 sens laterally (Fig. 10A).

Abd. I with seven ( $a_2$ –3,  $m_2$ –4,  $m_{2i}$ ,  $m_{4p}$ ) mac, 1+1 ms and 1+1 sens laterally (Fig. 10B).

Abd. II with two ( $m_3$ ,  $m_{3e}$ ) central and one ( $m_5$ ) lateral mac, 2+2 tric without modified chaetae, 1+1 sens laterally and 1+1 mic near internal tric (Fig. 10B).

Abd. III with two ( $a_2$ ,  $m_3$ ) central, and three ( $am_6$ ,  $pm_6$ ,  $p_6$ ) lateral mac, 3+3 tric without modified chaetae, 1+1 sens laterally, 1+1 mic near  $m_3$ , ms present (Fig. 10B).

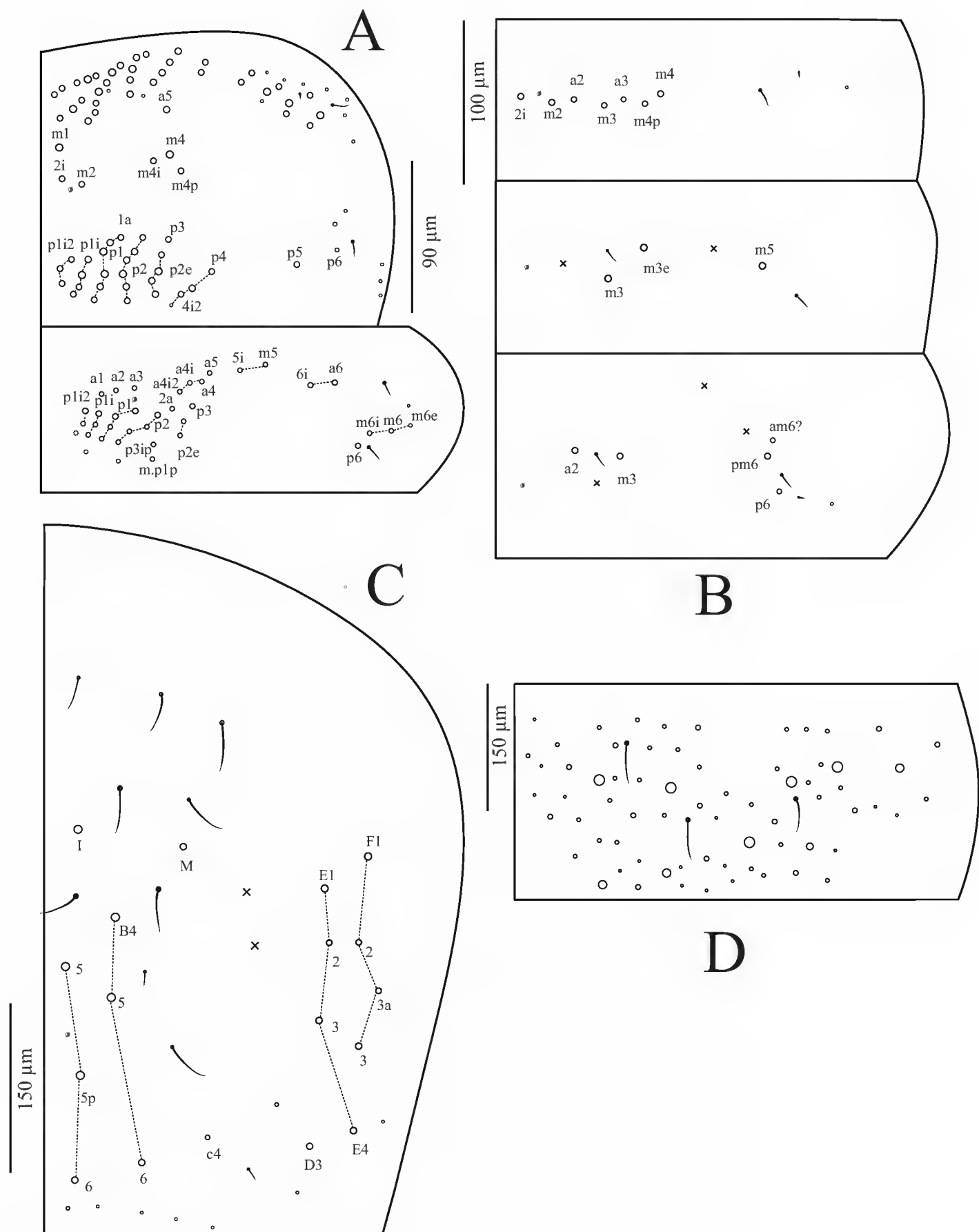
Abd. IV with eight central mac (I, M, A5–6, A5p, B4–6) and nine (D3, E1–4, F1–3, F3a) lateral mac, 2+2 tric and at least 8 long S-like chaetae, without modified chaetae (Fig. 10C).

Abd. V with at least 9 obvious mac mixed with several mes to small mac, and 3+3 sens (Fig. 10D). Abd. VI not analysed.

S-chaetae formula from Th. II to Abd. V: 2+ms, 2/1+ms, 2,2+ms,  $\approx 8$ , 3; as and ps sens on Abd. IV 1/3 as long as S-like chaetae (Figs 10A–D).

**Legs** (Figs 8J, K). Leg long; tita of leg III slightly longer than tita of legs I and II. Legs devoid of scales, covered with ordinary ciliated chaetae of various lengths, mic not seen. Trochanteral organ with 17–23 smooth, straight, unequal spine-like chaetae (Fig. 8K). The distal whorl of tita III with 9–10 subequal ciliated mes, irregularly arranged, and dorso-apical pointed tenent hair (claw II–III clavate in one specimen). A smooth, thin and long chaeta close to tenent hair absent. Vento-distal smooth chaeta of tita III thick, erected, pointed, rather short. Claw rather slender and elongated. Unguis of all claw with one inner tooth at 68 % from the base of claw; a pair of unequal basal teeth at about 50–52 % of inner edge from basis, outer tooth present at 40 % from base of the claw (Fig. 8J). Unguiculus approximately 2/3 as long as inner edge of the claw, pointed apically, devoid of inner and outer teeth (Fig. 8J).





**Figure 10.** *Coecobrya ellisi* sp. nov., continued **A** Chaetotaxy of dorsal Th. II– III **B** Chaetotaxy of dorsal Abd. I– III **C** Chaetotaxy of dorsal Abd. IV **D** Chaetotaxy of dorsal Abd. V.

**Ventral tube** (Figs 8H, I, 9C). Ventral tube about three times longer than wide. Lateral flaps with 7–8+7–8 chaetae (6–7+6–7 smooth chaetae and 0–2+0–2 ciliated chaetae present (Figs 8H–I, 9C). Anterior face with 6–7+6–7 ciliated chaetae, 2(3) of

them larger than others (Fig. 8H); posteriorly with 4 apical long smooth chaetae, and about 8–9 proximal smooth chaetae mixed with long and mic chaetae arranged roughly asymmetrically (Fig. 9C).

**Furcal complex** (Figs 9E–G). Tenaculum with four large teeth of decreasing size from the basal to the distal one of each ramus, on a prominent, irregular body, with a postero-basal strong serrated chaeta bent distally. Mucrodens 1.22–1.31 times longer than manubrium. Furcula without smooth chaetae. Manubrium with a dense cover of ciliated chaetae both dorsally and ventrally. Manubrial plaque with 2+2 psp and five to six ciliate chaetae (Fig. 9E). Distal part of manubrium ventrally with 10–12 + 10–12 ciliate chaetae (Fig. 9G). Dens without spines, annulated and covered with ciliated chaetae on both sides. Distal smooth part of dens longer than mucro. Mucro strong and falcate, basal spine long, nearly reaching the tip of the mucronal tooth (Fig. 9F).

**Genital plate.** Female genital plate with 2+2 genital mic, male genital plate not clearly seen.

**Ecology.** *Coecobrya ellisi* sp. nov. was found deep in a cave which has many chambers, on the muddy ground floor, on stalagmites and on wet rock walls. Many individuals were found feeding on the patches of bat guano in mesotrophic to eutrophic habitats. The relative humidity of the cave was 86–89% and the temperature was 25.8–27.5 °C. The cave where this species was found is located in an isolated limestone hill and has a narrow, vertical, entrance about 25 m deep which is equipped with iron ladders. The cave is approximately 250 m long and 30 m deep and many of the chambers are dug out and enlarged. Bad air, with a low oxygen level (<18%), was also detected in some chambers, including the one where the new species was found which is at the base of the iron ladders.

**Etymology.** This species is named in honor of Martin Ellis, a British speleologist who played a role in the Tham Luang cave rescue in Thailand (in 2018), and for his outstanding contribution to the study of cave fauna in Thailand. Our biological surveys have benefited tremendously from his support, including the discovery of this new species.

**Remarks.** *Coecobrya ellisi* sp. nov. is close to and shares most morphological characters with *C. phitsanulokensis* sp. nov. from Tham Yai Nakarat, Noen Mapang district, Phitsanulok province. The two caves where these new two species were discovered are only 55 km apart in a straight line. They are similar in chaetotaxy of dorsal head, number of sublobal hairs on maxillary outer lobe, labial chaetotaxy, medio-medial and medio-sublateral mac on Th. II, number of mac on Abd. I, Abd. III, pointed tenent hair, anterior face of ventral tube and number of chaetae on manubrium plaque. However, *C. ellisi* sp. nov. can be easily distinguished from *C. phitsanulokensis* sp. nov. by the number of An mac on dorsal head (3 vs. 5), number of central mac on Abd. II (2 vs 3(4), number of central mac on Abd. IV (8 vs. 9), lateral mac on Abd. IV (9 vs. 8), number of inner teeth of claw (3 vs. 2) and number of chaetae on ventro-distal part of manubrium (10–12 vs. 13) (Table 1). Moreover, *C. ellisi* sp. nov. possesses orange dots clearly pigmented on the antennae, head, body, legs and manubrium while all other troglomorphic *Coecobrya* species so far described in Thailand are devoid of any trace of pigmentation (Fig. 1C).



***Coecobrya phitsanulokensis* Jantarit & Nilsai, sp. nov.**

<http://zoobank.org/04257381-2AD3-4350-B626-019EFF23903F>

Figures 1D, 11–13, Table 1

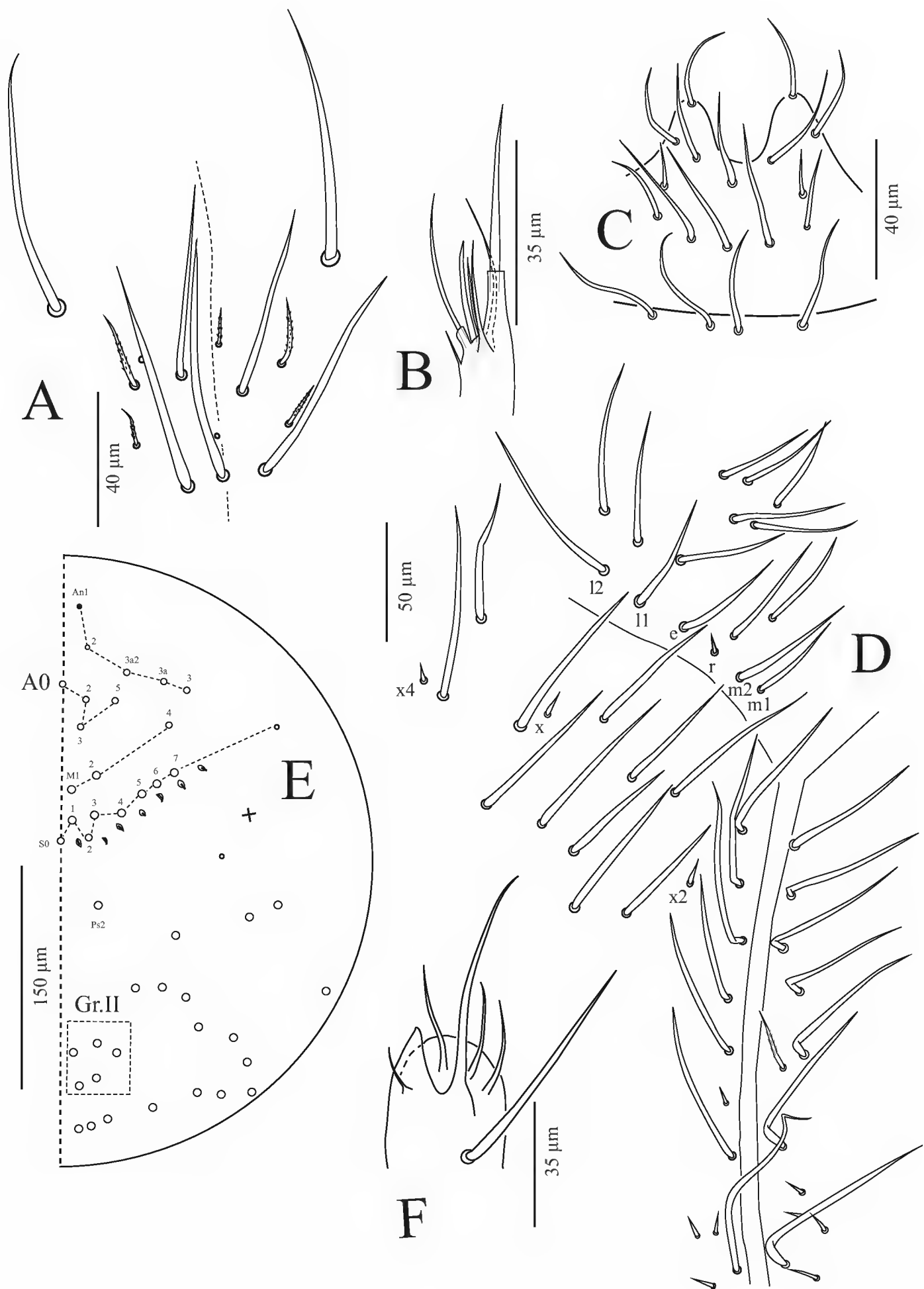
**Type material.** *Holotype*: female on slide. Thailand, Phitsanulok province, Noen Mapang district, Tham Yai Nakarat, altitude 85 m a.m.s.l., 16.5052°N, 100.6864°E. 7.X.2019; S. Jantarit, A. Nilsai, K. Sarakhamhaeng and K. Jantapaso leg. (sample # THA\_SJ\_PLK01), dark zone of a cave, by entomological aspirator. *Paratypes*: same data as holotype, 7 specimens (3 females and 4 subadults on slides). *Additional material*: same data as holotype, 13 specimens (in ethanol)

Holotype and seven paratypes on slides deposited in NHM-PSU.

**Description.** *Habitus* (Fig. 1D). Medium size Entomobryidae. Body length 1.8–2.3 mm (holotype 1.8 mm). No scales. Eyes absent. Color: whitish in alcohol, without pigmentation. Four antennal segments. Body slender not bent nor humped at the level of Th. II. Th. II slightly larger than Th. III. Abd. IV 3.58–3.88 times as long as Abd. III along the dorsal midline.

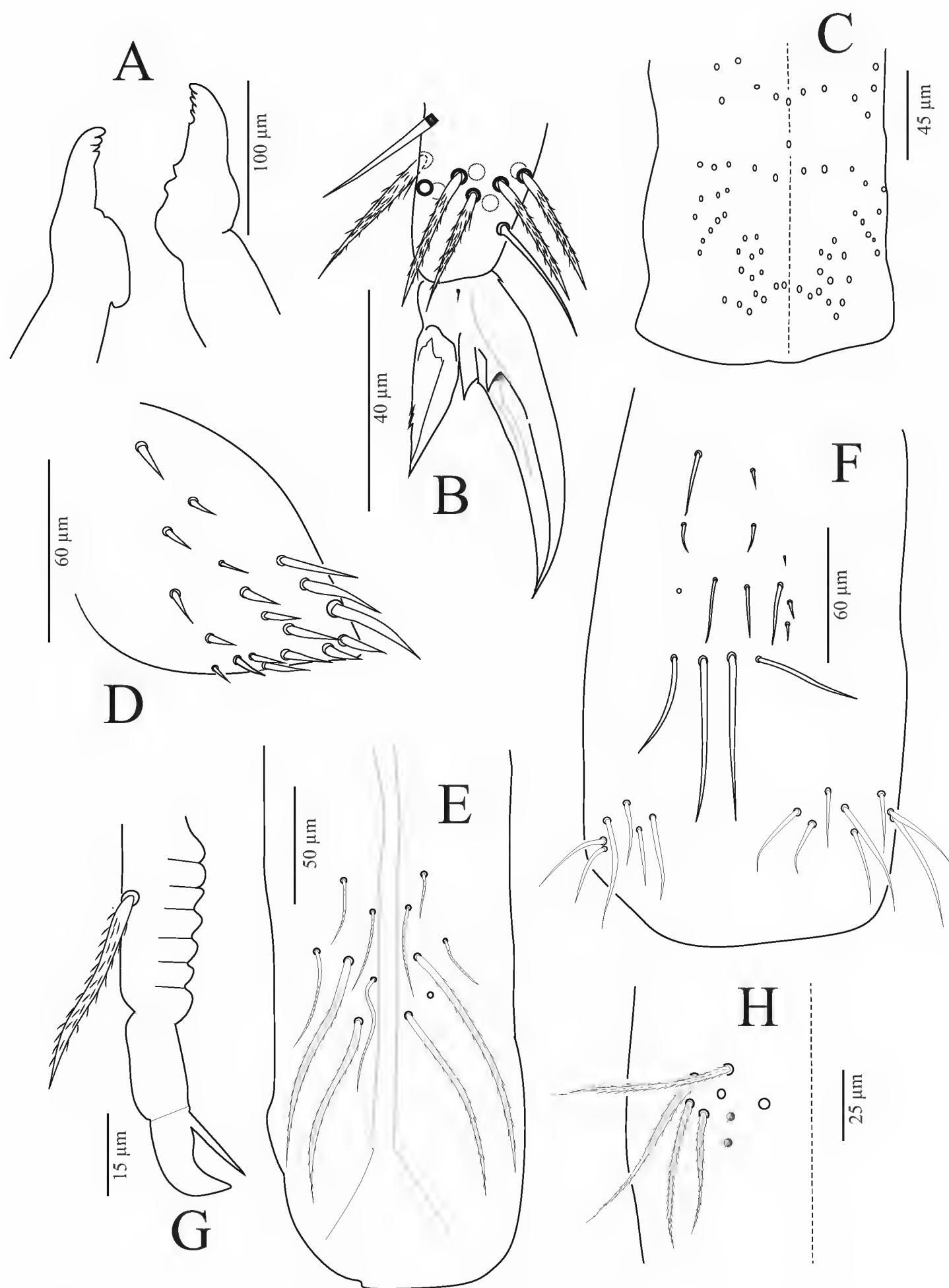
*Pseudopores* (Figs 12H, 13A, B, D). Pseudopores present as round flat disks, smaller than mac sockets, except for the coxae and manubrium where psp are as large as mac sockets, present on various parts of the body: antennae, head, tergites, coxae and manubrium. On antennae, psp located ventro-apically between the tip of antennal segments and the chaetae of the apical row, or just below the apical row of chaetae (2 psp on Ant. I, 2–3 psp on Ant. II, and 3 psp on Ant. III). On the head, 1–2 psp located externally on each peri-antennal area. On tergites, 1+1 psp close to the axis from Th. II to Abd. IV (Figs 13A, B, 13D). On coxae, 1–2 psp on coxae I, 2–3 psp on coxae II and 1–2 psp on coxae III, located close to longitudinal rows of chaetae. On manubrium, 2+2 dorso-apical ones (Fig. 12H).

*Clypeus and mouthparts* (Figs 11A–C, F, 12A). Clypeal area with three long, smooth prefrontal chaetae; 9 middle chaetae (two long smooth chaetae, 7 small ciliated chaetae from mic to mes arranged asymmetrically), and two long, smooth lateral chaetae (Fig. 11A). Prelabral and labral chaetae 4/5, 5, 4, all thin and smooth; three median chaetae of the first and second rows longer than the two lateral ones (32–38 vs. 12–15 µm) (Fig. 11C). Distal border of the apical non-granulated area of the labrum with a relatively narrow median U- or V-form intrusion into the granulated area dorsally; apical edge without spines (Fig. 11C). Ventro-distal complex of labrum well differentiated, asymmetrical, with 1+1 distal combs 15–16 minute on the right side and 13 strong and larger teeth on the left side, and an axial pair of long sinuous tubules. Maxillary outer lobe with one basal chaeta, one apical chaeta (basal chaeta thicker than apical one) and four smooth sublobal hairs (65–70 vs. 25–32 µm) (Fig. 11F). Labial palp strongly modified for the genus, with 0, 5, 0, 4, 4 guards for papillae A–E. Lateral process of labial palp subcylindrical, as thick as normal chaetae, with tip beyond the apex of the labial papilla (Fig. 11B). Mandible apex blunt



**Figure 11.** *Coecobrya phitsanulokensis* sp. nov. **A** Clypeal chaetae **B** Labial palp **C** Prelabral and labral chaetae **D** Anterior side of ventral tube **E** Dorsal cephalic chaetotaxy **F** Outer maxillary lobe.





**Figure 12.** *Coecobrya phitsanulokensis* sp. nov., continued **A** Mandibles **B** Distal part of tita III and claw complex **C** Distal part of manubrium ventrally **D** Trochanteral organ **E** Anterior side of ventral tube **F** Posterior side of ventral tube and Lateral flap **G** Mucro **H** Manubrium plaque.

and strong, asymmetrical (left with four teeth, right with five teeth); molar plate with three strong pointed basal teeth, and 3–(5) smaller inner distal teeth, identical in both mandibles (Fig. 12A). Maxilla capitulum with a three-toothed claw and several stout ciliated lamellae; lamella 2 large and broad, lamella 3 well developed; several other lamellae present.

**Antennae.** Antennae long, approximately 3.1–4.2 times as long as cephalic diagonal. Antennal segments ratio as I: II: III: IV. 1: 0.5–0.73: 0.48–0.7: 0.41–0.68 (N = 6). Antennal segments not subdivided nor annulated. Antennal chaetal types not analyzed in detail. Ant. I ventrally with many smooth spiny mic of various sizes in its basal part, many subcylindrical, hyaline sens in its middle to apical part, and many long smooth straight chaetae. The paddle-like chaetae on Ant II absent. Ant. III organ with five sens not clearly seen in all specimens. Ant. IV without apical bulb. Subapical organite not distinctly knobbed, swollen, slightly enlarged apically, inserted dorsally.

**Dorsal head chaetotaxy** (Fig. 11E). Dorsal cephalic chaetotaxy with five antennal chaetae (An1–3, An3a2, An3a, An1 as mes), four anterior mac (A0, A2–3 and A5) three median (M1, M2 and M4) and eight sutural mac (S0, S1–S7); Gr. II with 4 or 5 mac; A<sub>0</sub> as mac; 5–7+5–7 scale-like structures present below sutural mac, probably inside the integument; a pair of short cephalic trichobothria, external and close to the middle of the head (Fig. 11E).

**Ventral head chaetotaxy** (Fig. 11D). Chaetae of labial basis all smooth ( $m_1m_2rel_1l_2$ ), ( $m_1mrel_1l_2$  sensu Zhang and Pan, 2020), chaetae  $m_1(m_1)$ ,  $e$  and  $l_1$  subequal,  $r$  thin and shortest, and  $l_2$  longest,  $m_2$  longer and thicker than  $m_1$ . The ratio of  $r$  to  $m_2$ : 0.13–0.19 (Fig. 11D). Postlabial chaetae  $X_2$ ,  $X$  and  $X_4$  minute chaetae,  $X_1$  and  $X_3$  absent. On each side of cephalic groove with 8–11 chaetae, of which the anterior five always long and smooth, others either smooth or finely ciliated, at least 3–4 minute chaetae always present on the posterior ones (Fig. 11D).

**Tergite chaetotaxy** (Figs 13A–D). Th. II with three ( $m_1$ ,  $m_2$ ,  $m_{2i}$ ) medio-medial, three ( $m_4$ ,  $m_{4p}$ ,  $m_{4i}$ ) medio-sublateral and 31–33 posterior mac; 1+1 ms and 2+2 sens antero-laterally (Fig. 13A).

Th. III with 32–33 mac. 2+2 sens laterally (Fig. 13A).

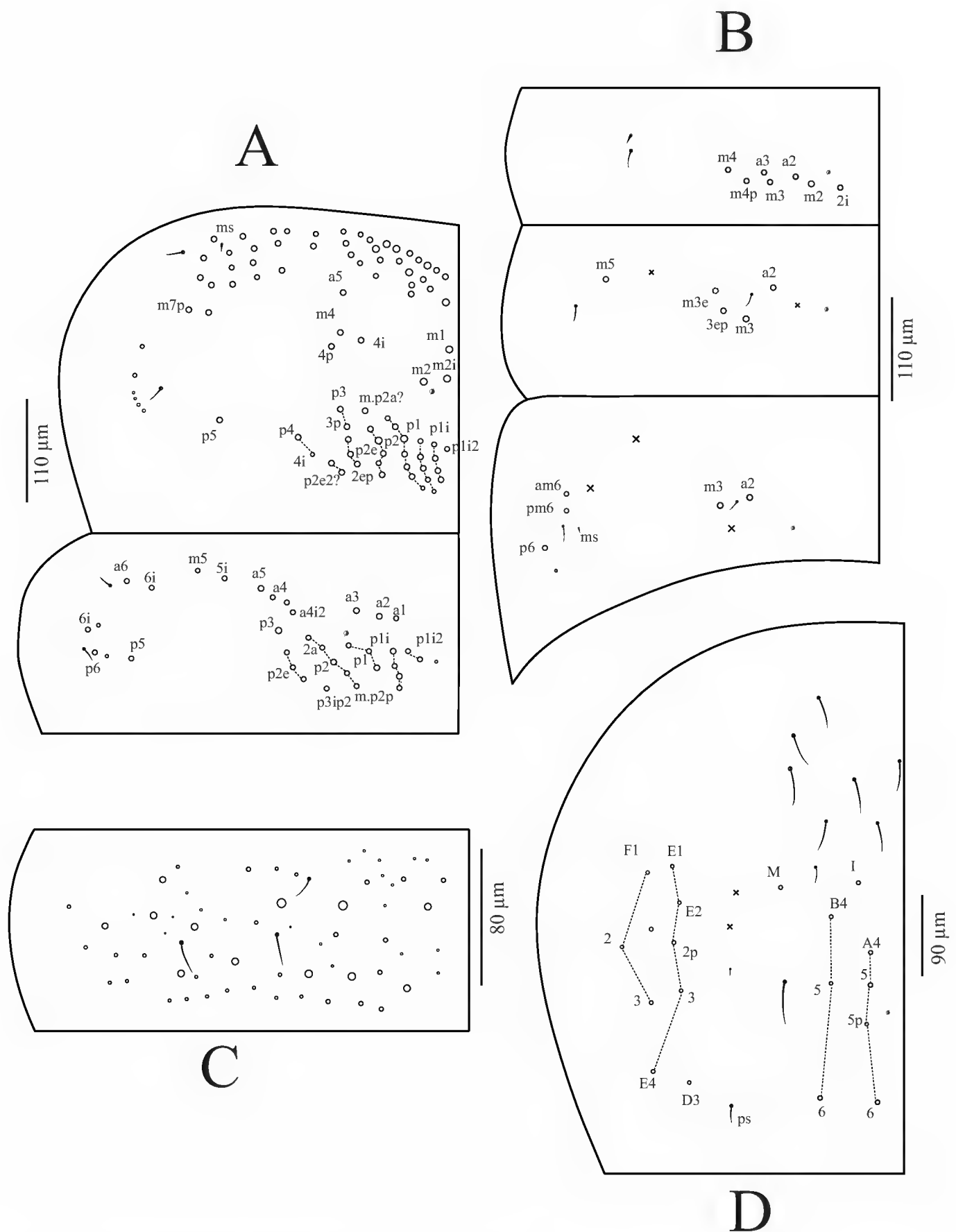
Abd. I with seven ( $a_2$ –3,  $m_4$ ,  $m_2$ –3,  $m_{2i}$ ,  $m_{4p}$ ) mac, 1+1 ms and 1+1 sens laterally (Fig. 13B).

Abd. II with 3(4) ( $a_2$ ,  $m_3$ ,  $m_{3ep}$ , and  $m_{3e}$  sometimes present) central and one ( $m_5$ ) lateral mac. 2+2 tric without modified chaetae, 1+1 sens laterally and 1+1 mic near internal tric (Fig. 13B).

Abd. III with two ( $a_2$ ,  $m_3$ ) central and three ( $am_6$ ,  $pm_6$ ,  $p_6$ ) lateral mac, 3+3 tric without modified chaetae, 1+1 sens laterally, 1+1 mic near  $m_3$ , ms present (Fig. 13B).

Abd. IV with nine central mac (I, M, A4–6, A5p, B4–6) and nine (D3, E1–4, E2p, F1–3) lateral mac, 2+2 tric and about 8 long S-like chaetae, without modified chaetae (Fig. 13D).





**Figure 13.** *Coecobrya phitsanulokensis* sp. nov., continued **A** Chaetotaxy of dorsal Th. II–III **B** Chaetotaxy of dorsal Abd. I–III **C** Chaetotaxy of dorsal Abd. V **D** Chaetotaxy of dorsal Abd. IV.

Abd. V with 12 obvious mac mixed with several mes to small mac, and 3+3 sens (Fig. 13C). Abd. VI not analysed.

S-chaetae formula from Th. II to Abd. V: 2+ms, 2/1+ms, 2, 2+ms, ≈8, 3; as and ps sens on Abd. IV 1/3 as long as S-like chaetae (Figs 13A–D).

**Legs** (Figs 12B, D). Leg long; tita of leg III slightly longer than tita of legs I and II. Legs devoid of scales, covered with ordinary ciliated chaetae of various lengths, mic not seen. Trochanteral organ with 19 smooth, straight, unequal spine-like chaetae (Fig. 12D). The distal whorl of tita III with 10 subequal ciliated mes, irregularly arranged, and dorso-apical pointed tenent hair. A smooth, thin and long chaeta close to tenent hair absent. Ventro-distal smooth chaeta of tita III thick, erected, pointed, rather short. Claw slender and elongated. Unguis of all claws without inner tooth, and a pair of subequal basal teeth at about 29–36 % of inner edge from basis. Unguiculus approximately 1/2 as long as the inner edge of the claw, rather swollen basally, pointed apically, devoid of inner tooth, with at least 3–4 minute outer teeth, often inconspicuous, at 3/4 of its length (Fig. 12B).

**Ventral tube** (Figs 12E, F). Ventral tube about four times longer than wide. Lateral flaps with 6–8+6–8 smooth chaetae (Fig. 12F). Anterior face with 6–7+6–7 ciliated chaetae, four of them larger than others (Fig. 12E); posterior face with 4 long, smooth apical chaetae and about 11 smooth chaetae arranged roughly asymmetrically, mixed with mic or small acuminate mes (Fig. 12F).

**Furcal complex** (Figs 12C, G, H). Tenaculum with four large teeth of decreasing size from the basal to the distal one of each ramus, on a prominent, irregular body, with a postero-basal strong serrated chaeta bent distally. Mucrodens 1.11–2.27 times longer than manubrium. Furcula without smooth chaetae. Manubrium with a dense cover of ciliated chaetae both dorsally and ventrally. Manubrial plaque with 2+2 psp and five to six ciliate chaetae (Fig. 12H). Distal part of manubrium ventrally with 13–15+13–15 ciliate chaetae (Fig. 12C). Dens without spines, annulated and covered with ciliated chaetae on both sides. Distal smooth part of dens slightly longer than mucro. Mucro strong and falcate, basal spine long, nearly reaching the tip of the mucronal tooth (Fig. 12G).

**Genital plate.** Female genital plate with 2+2 genital mic.

**Ecology.** *Coecobrya phitsanulokensis* sp. nov. was found from the twilight zone (ca. 20 m from the cave entrance) to the dark zone of the cave on wet and muddy ground and on the decaying organic material inside the cave. The length of the main passage in the cave is about 300 m. The temperature was 26.2–27.9 °C, the soil temperature was 24.1–24.3 °C and the relative humidity in the cave was 78–89 %. The cave has a seasonal stream in it, but there was no water during our visit. This cave is developed in a very small isolated limestone hill (0.4 × 0.9 km) surrounded by a flood plain agricultural landscape, with at least seven other caves having been reported in this hill.

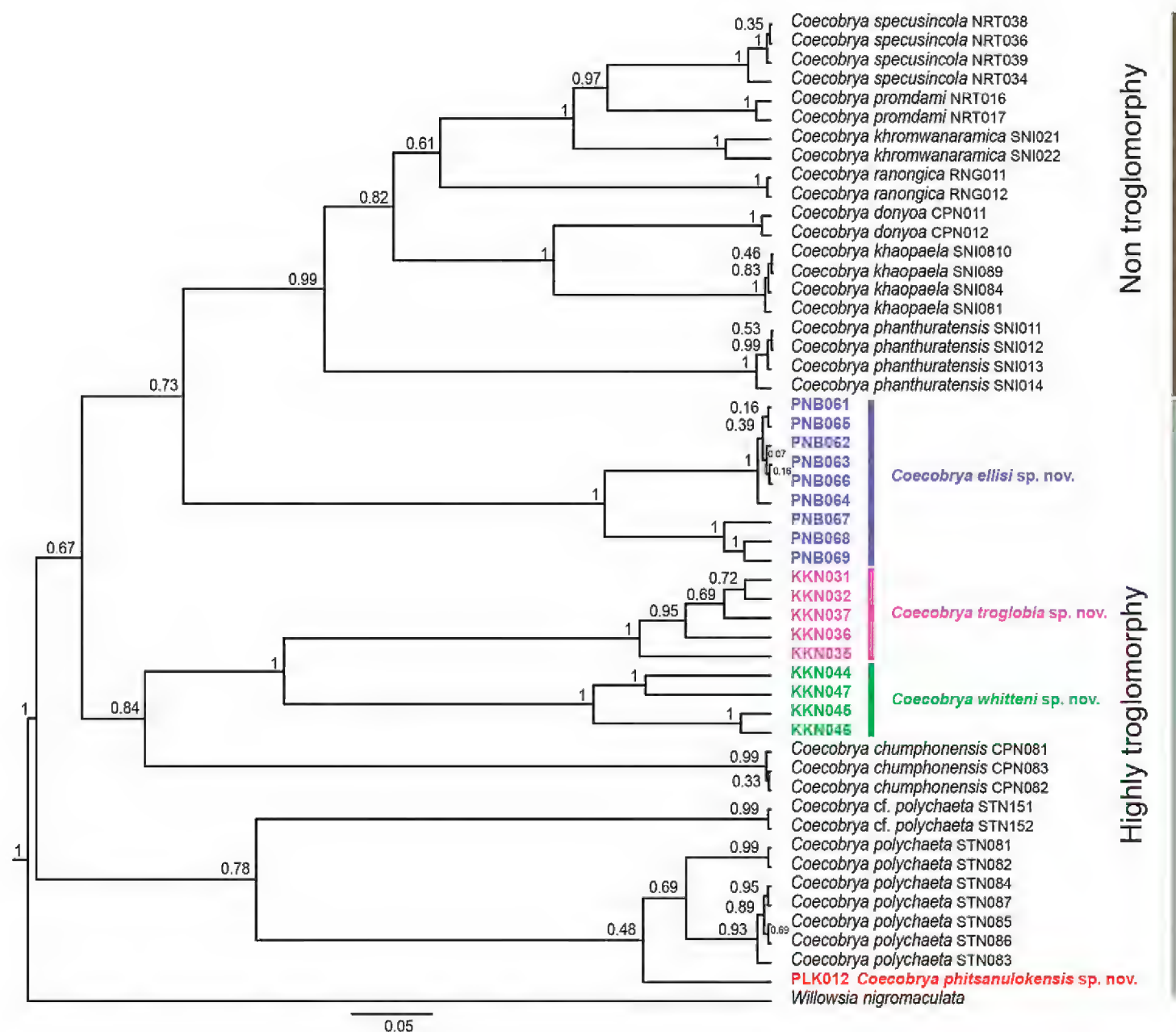
**Etymology.** This species is named after the type locality, Phitsanulok province, where the material was collected.

**Remarks.** Among the troglobitic *Coecobrya* species *C. phitsanulokensis* sp. nov. is similar to *C. ellisi* sp. nov. from Tham Tho, Nong Phai district, Phetchabun province. For the species diagnosis see the remarks under *Coecobrya ellisi* sp. nov. and Table 1.

## Genetic distances and phylogenetic analyses

The pairwise interpopulation distances of the four new species were 10.50–23.70% for COI, 24.11–43.33% for 16S, and 7.90–36.80% for 28S. Considering COI alone which is a favorable/typical DNA barcoding used for species identification, delimitation and discovery, the pairwise interpopulation distances between *C. whitteni* sp. nov. and *C. troglobia* sp. nov., which are most similar in morphology, was 10.60–11.40%. The distance between *C. whitteni* sp. nov. and *C. ellisi* sp. nov. was 20.90–23.20% and between *C. troglobia* sp. nov. and *C. ellisi* sp. nov. was 21.40–23.70% (Suppl. material 2, Table S2).

Both Bayesian inference (BI) and Maximum likelihood (Suppl. material 1) analyses provided mostly congruent gene tree topologies (Fig. 14, Suppl. material 1), with the four described species forming their own distinct clades. The tree topology cor-



**Figure 14.** Bayesian tree (BI) of *Coecobrya* species from Thailand based on the concatenated dataset (COI, 16S, and 28S sequences). Posterior probabilities are provided on the branches, and the four new species are in bold with different colors.



responds to the morphological characterization of the studied *Coecobrya* species and many branches received maximum support value of both methods (posterior probability PP = 1, bootstrap ML = 99–100, Fig. 14, Suppl. material 1). All non-troglomorphic species were clustered within the same clade with maximum support value (PP = 0.99), while all troglomorphic *Coecobrya* constituted a paraphyletic group.

## Discussion

It is widely accepted that COI is the standard DNA barcode and the first choice for species identification, while 16S and 28S rDNA are used as complementary genes (Hebert et al. 2003; Porco et al. 2014; Zhang et al. 2018b). Unfortunately, we failed to amplify the COI sequence from *C. phitsanulokensis* sp. nov., making it impracticable for COI comparison with other species. Only 16S and 28S sequences were successfully sequenced.

The results of the molecular approach supported the results of morphological species discrimination in separating the four nominal populations as valid species. The genetic distances between the populations clearly exceed the standard thresholds of 2% (Hebert et al. 2003), 10% (Rougerie et al. 2009) or even 14% (Porco et al. 2014), supporting the separation of the four newly described species. According to the BI (Fig. 14), all seven non-troglomorphic species (*C. donyoo*, *C. khaopacla*, *C. khromwanaramica*, *C. phanthuratensis*, *C. promdami*, *C. ranongica* and *C. specusincola*) form a monophyletic group with high support value (PP=0.99) indicating their closely relationship based on their geographical radiation in southern Thailand. All troglomorphic *Coecobrya* in Thailand, especially the four new described species, are clearly separated from other troglomorphic species found in Thailand, however, they do not form as a monophyletic group. This is not exceptional and is expected due to convergent selective pressure of cave species depending on the microhabitat preferences.

Regarding their systematic position, *C. phitsanulokensis* sp. nov. (central Thailand) was recovered as the sister clade to *C. polychaeta* (southern Thailand) which is unexpected and raises a question on the evolutionary relationship, mainly because both species are 1,078 km apart from each other in a straight line. Their morphological characters are also largely different (Table 1). In fact, *C. phitsanulokensis* sp. nov. is morphologically most similar to *C. ellisi* sp. nov. (Table 1) and these species are only 55 km apart in a straight line from each other. Therefore, the phylogenetic relationship between *C. phitsanulokensis* sp. nov. and *C. ellisi* sp. nov. is expected to be closely related. This is obvious in the case of *C. whitteni* sp. nov. and *C. troglobia* sp. nov. where their morphological characters are similar and they are sister groups in our phylogenetic reconstruction (Fig. 14). The placement of *C. phitsanulokensis* sp. nov. with *C. polychaeta*, however, is supported by a low Bayesian value (PP = 0.48, Fig. 14), but they are sister groups with *C. whitteni* sp. nov., *C. troglobia* sp. nov. and *C. chumphonensis* in ML (Suppl. material 1), indicating the uncertainty of relationships in the clade. This could be due to the lack of COI sequence in *C. phitsanulokensis* sp. nov. with only a single sequence of 16S and 28S sequences suggesting the

need for the COI sequence and additional numbers of representative samples, which would enable the resolution of the phylogenetic relationships.

It is widely accepted that the troglomorphic Collembola prefer oligotrophic habitats with wet and moist environments (Deharveng and Bedos 2012; Deharveng et al. 2018; Lukić et al. 2018; Lukić 2019; Nilsai et al. 2017; Jantarit et al. 2019). However, this cannot be applied to tropical areas where some troglomorphic species are able to reside in low humidity. This is evident in *C. troglobia* sp. nov. where the population of this new species is found in a rather dry habitat with humidity lower than 75 %, whereas most of the troglomorphic *Coecobrya* species described so far in Thailand favor wet and moist habitats (85–99.9 % humidity). It is generally recognized that subterranean terrestrial invertebrates, especially the highly troglomorphic ones, are particularly sensitive to microclimatic variations, especially microclimatic changes (Nicolosi et al. 2021). Therefore, the presence of *C. troglobia* sp. nov. in the low humidity inside the cave is not accidental and could be further explained by two hypotheses: 1) they are tolerant to environmental change to a considerably lower humidity or 2) troglomorphic *Coecobrya* can also be well-adapted in the subterranean environment where humidity is low. This remains to be investigated in more detail.

Regarding the distribution range inside Thai caves, all non-troglomorphic species described so far always have a wide range of dispersion, from the entrance to deep inside the cave and all are always associated with eutrophic environments, especially with large amounts of animal feces, such as bat guano, and plant debris. Their populations are obviously abundant with many thousands of individuals in each cave. In contrast, all troglomorphic species have a narrow dispersion in the cave and are highly restricted to a chamber or chambers where it has been collected. Their populations are small to scarce. They are usually associated with an oligotrophic environment. Interestingly, some species are also linked to the mesotrophic to eutrophic habitats. This is apparent in the case of *C. whitteni* sp. nov. and *C. ellisi* sp. nov. where many individuals were collected in areas where a large number of guano piles were present, indicating that troglomorphic *Coecobrya* in tropical areas are not always restricted to the oligotrophic habitat and they are also able to live or search for food in mesotrophic to eutrophic habitats where energy supply is high. This is contrary to the assumption that high-energy habitats are only exploited by non- or weakly troglomorphic species (Deharveng and Bedos 2012). An increase in taxonomic surveys and the discovery of more highly troglomorphic *Coecobrya* species on a larger scale would significantly confirm their ecological preferences in caves.

This study indicates that the genus *Coecobrya* in Thai caves is very diversified, highly restricted and endemic to a specific cave. This is well-explained in the case of *C. whitteni* sp. nov. and *C. troglobia* sp. nov. where both species share most morphological characters (Table 1) and the two species are only 3.4 km distant in a straight line from each other. The BI and ML methods confirm the separation of both species with a maximum support value (Fig. 14, Suppl. material 1). This implies that the morphological characters used to separate both species, i.e. number of chaetae along cephalic ventral groove on head, dorsal head and body chaetotaxy, have important taxonomic information for spe-

cies discrimination (Nilsai et al. 2017; Zhang et al. 2011b, 2018a; Jantarit et al. 2019). Our findings could be further explained by the result of allopatric speciation by limestone outcrop which may play a crucial role in disconnecting the gene flow between the populations. Also, the subterranean environments of both caves have their own unique microhabitats which favor the independent evolutionary adaptation of each species.

### Key to the troglomorphic *Coecobrya* of Thailand

This key is modified from Jantarit et al. (2019) which includes all troglomorphic *Coecobrya* species of Thailand. All species have long antennae more than 2.5 times as long as the cephalic diagonal.

- 1 Outer maxillary lobe with 3 sublobal hairs; a single chaeta m on labium .... **2**
- Outer maxillary lobe with 4 sublobal hairs; two chaetae m on labium ..... **5**
- 2 Labial chaetae as mRel<sub>1</sub>l<sub>2</sub>; dorsal head with 4+4 M chaetae and Gr. II with 6–7 chaetae..... ***C. chumphonensis* Zhang & Nilsai, 2017**
- Labial chaetae as mrel<sub>1</sub>l<sub>2</sub>; dorsal head with 3+3 M chaetae and Gr. II with 4 chaetae..... **3**
- 3 Claw without unpaired inner tooth; central mac of Abd. II with 3 mac; central mac of Abd. IV with 7–9 mac ..... ***C. cavicta* Nilsai & Zhang, 2017**
- Claw with unpaired inner tooth; central mac of Abd. II with 2 mac; central mac of Abd. IV ≤ 5 mac..... **4**
- 4 Chaetae An of dorsal head with 4 mac; medio-medial mac of Th. II with 2 mac; medio-sublateral mac of Th. II with 1 mac ..... ***C. whitteni* sp. nov.**
- Chaetae An of dorsal head with 5 mac; medio-medial mac of Th. II with 3 mac; medio-sublateral mac of Th. II with 2 mac ..... ***C. troglobia* sp. nov.**
- 5 Antennae >8 times as long as cephalic diagonal; tita with a dorso-distal smooth chaeta in addition to the tenent hair; claw very slender ..... ***C. sirindhornae* Jantarit, Satasook & Deharveng, 2019**
- Antennae 3–7 times as long as cephalic diagonal; tita without a dorso-distal smooth chaeta in addition to the tenent hair; claw moderately slender..... **6**
- 6 Chaeta A0 of dorsal head as mic; Gr.II with 3 chaetae; medio-media on Th. II with 6 or 7 mac; mac on Abd. I with 8–9 mac; central mac of Abd. III with 1 mac..... ***C. polychaeta* Zhang & Nilsai, 2017**
- Chaeta A0 of dorsal head as mac; Gr.II with 4 or 5 chaetae; medio-media on Th. II with 3 mac; mac on Abd. I with 7 mac; central mac of Abd. III with 2 mac..... **7**
- 7 Number of central mac on Abd. II with 2 mac; central mac of Abd. IV with 8 mac; claw with unpaired inner tooth..... ***C. ellisi* sp. nov.**
- Number of central mac on Abd. II with 3 or 4 mac; central mac of Abd. IV with 9 mac; claw without unpaired inner tooth ..... ***C. phitsanulokensis* sp. nov.**



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## Supplementary material I

### Maximum likelihood phylogenetic tree (ML)

Authors: Areeruk Nilsai, Matsapume Detcharoen, Nerivania Nunes Godeiro, Sopark Jantarit

Data type: ML phylogenetic tree

Explanation note: Maximum likelihood phylogenetic tree (ML) of *Coecobrya* species from Thailand based on the concatenated dataset (COI, 16S, and 28S sequences).

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Link: <https://doi.org/10.3897/subtbiol.41.76926.suppl1>

## Supplementary material 2

### Table 1–3. Primers, genetic distance and Genbank accession numbers

Authors: Areeruk Nilsai, Matsapume Detcharoen, Nerivania Nunes Godeiro, Sopark Jantarit

Data type: molecular data

Explanation note: **Table S1.** Primers used for amplification and sequencing of the COI, 16S and 28S. **Table S2.** Inter/intraspecific genetic divergences for COI, 16S and 28S markers; Abbreviations for the code of four new species: KKN03, *Coecobrya troglobia* sp. nov.; KKN04, *C. whitteni* sp. nov.; PNB, *C. ellisi* sp. nov.; PLK01, *C. phitsanulokensis* sp. nov. **Table S3.** The sequenced terminals, and Genbank accession numbers used in this study.

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